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(54) Title: MAMMALIAN elk POTASSIUM CHANNEL GENES

(57) Abstract

The present invention is directed to the isolation, cloning and sequencing of mammalian elk1, elk2, and EAG2 K channel genes. The amino acid sequences of the expressed proteins are also provided. Information on the distribution of the gene and their expression products in various relevant nervous system tissue is also provided. The gene and protein sequences and the distribution pattern information is helpful in the research and development of pharmaceuticals with specific action on the different K channels.

MAMMALIAN *elk* POTASSIUM CHANNEL GENES

FIELD OF THE INVENTION

The present invention relates to the isolation,
cloning and sequencing of genes and the encoded proteins for
voltage-gated potassium channels (K channels). More
particularly, the invention is directed to the isolation,
cloning and sequencing of *eag* and *eag-like-K+* channel genes
(*elk*).

The availability of these genes provide the tools for
further reasearch into the physiobiological characteristics of
the various genes and proteins for K channels; the development
of medicines which are effective for treating the specific
disease conditions arising from mutations or defects in the
specific K channels and the screening of drugs to ensure that
the elk potassium channels will not be blocked. The
availability of the *elk* gene/elk protein ensures that drugs
screened for the treatment of other diseases will not affect
the physiological functions controlled by the *eag* and *elk*
potassium channel gene.

BACKGROUND OF THE INVENTION

Potassium channels (K channels) play a major role in a
wide range of cellular functions, including electrical
excitability and synaptic plasticity in neurons. K channels
are involved in determining and stabilizing the resting
membrane potential, the re-polarization of action potentials,
control of firing patterns and modulation of neurotransmitter
release of nerve cells. Some of the functions controlled are
basic, such as the setting of the resting potential; and some
of the functions are very specific, such as defining the
interval between spikes of endogenously beating cells. These

functions are involved in a number of physiological processes, including regulation of heartbeat, dilation of arteries, release of insulin, excitability of nerve cells, and regulation of renal electrolyte transport. Potassium channels

5   are thus found in a wide variety of animal cells such as nervous, muscular, glandular, immune, reproductive, and epithelial tissue. These channels allow the flow of potassium ions in and/or out of the cell under certain conditions. For example, the outward flow of potassium ions upon opening of

10   these channels makes the interior of the cell more negative, counteracting de-polarizing voltages applied to the cell. These channels are regulated, e.g., by calcium sensitivity, voltage-gating, second messengers, extracellular ligands, and ATP-sensitivity. It is important for the potassium channels

15   to be operating properly to maintain healthy functions in the body. It is, therefore important that medicines for the treatment of diseases unrelated to the function of the ion channels do not adversely affect the proper function of these channels.

20   It is known that a variety of genes encode the proteins of the K channels with diverse electrophysiological and pharmacological properties. H. Terlau et al., _Pflughers Arch._, (1996) 432:301-312. The research is ongoing to determine the number of the genes involved and the

25   characteristics and functions of the proteins which the genes encode.

        Based on recent molecular cloning studies, a hierarchical classification scheme has been developed. Sodium, calcium and potassium channels belong to a

30   'superfamily' of ion channels.

        Structurally, K channels can also be grouped into

three main classes: six-transmembrane (6TM), four-transmembrane (4TM) and two-transmembrane (2TM) subunits. The 6TM superfamily may be further divided into six families, among which are the voltage-gated, KQT-like, *EAG*-like, Slo-

5   like, cyclic nucleotide-gated and "SK" channels. Each of the families may be further subdivided into "subfamilies". For example, in the voltage-gated family, there are four subfamilies: Shaker, Shab, Shaw and Shal, corresponding to Kv1, Kv2, Kv3 and Kv4, respectively. Between subfamilies of

10   the 6TM family, the observed amino acid sequence homology is about 40%. A higher degree of homology, 50%-70%, is shared among members of a single subfamily of the 6TM family. This is true intra or inter species. Whereas, in the 2TM and 4TM families, much less conservation is observed across species.

15   A schematic of the classification scheme is shown in Fig.1. A. Wei et al., supra.

Potassium channels are typically formed by four alpha subunits, and can be homomeric (made of identical alpha subunits) or heteromeric (made of two or more distinct types

20   of alpha subunits). In addition, potassium channels made from Kv, KQT and Slo subunits have often been found to contain additional, structurally distinct auxiliary, or beta, subunits. These subunits do not form potassium channels themselves, but instead they act as auxiliary subunits to

25   modify the functional properties of channels formed by alpha subunits. For example, the Kv beta subunits are cytoplasmic and are known to increase the surface expression of Kv channels and/or modify inactivation kinetics of the channel (Heinemann et al. J. Physiol 493:625-633 (1996); Shi et al.,

30   Neuron 16(4):843-852 (1996)). In another example, the KQT family beta subunit, minK, primarily changes activation kinetics(Sanguinetti et al., Nature 384:80-83 (1996)).

Voltage-gated K channels are encoded by two groups: the extended Shaker family including the Ca^{2+}-activated SK and BK channels and those ion channels with a cyclic nucleotide-binding domain. The latter include the cyclic nucleotide-gated channels, the plant inward-rectifying channels and the *ether-a-go-go* (EAG) channels. H. Terlau et al., J. Physiol., (1997) 502.3:537-543. The *eag*-like family comprise three related voltage-gated K channel genes. The first of the family, the *eag gene*, was initially identified in *Drosophila*. J. Warmke *et al.*, Science (1991) 252:1560-1562. Two related *Drosophila* genes were subsequently described: the *eag*-related-gene (*erg*) and the *eag*-like-K^+ channel gene (*elk*). Warmke and Ganetzky, PNAS, USA (1994) 91: 3438-3442. Mammalian homologues of the *eag* genes have been identified and the potassium channels that they encode have been expressed and characterized. Warmke and Ganetzky (1994), supra; J. Ludwig *et al.*, EMBO J. (1994) 13:4451-4458; G.A. Robertson *et al.*, Neuropharmacol. (1996) 35:841-850; and W. Shi *et al.*, J Neurosci. (1997) 17:9423-9432.

Up to late 1997, only a single representative of the *erg* family, human *erg* (Herg) was found. There is considerable interest in the *erg* gene because mutations were shown to underlie one form of a human genetic disease known as long QT (LQT) syndrome, which gives rise to cardiac arrhythmia and an increase incidence of sudden death.

In *Xenopus* oocytes, Herg forms voltage-sensitive K channels with a distinctive inactivation mechanism that attenuates efflux of K+ during depolarization (Sanguinetti et al., Cell (1995) 81, 299-307. The *erg* channels most closely resemble those of native cardiac I_{Kr} channels. Through tissue distribution studies, it was found that the Herg gene is expressed abundantly in the nervous system and heart muscle.

Up to the present three members of the mammalian *erg* family have been cloned and sequenced by the inventors and reported in W. Shi et al, <u>J. Neurosci.</u> (1997) 17:9423-9432.

5   The rat *eag*1 has also been identified, cloned and sequenced. No other *eag* gene has been identified, or cloned. To date, no mammalian homologue of the *Drosophila elk* gene has been described, nor have the biophysical properties of any *elk* channel been characterized.

OBJECTIVES OF THE INVENTION

10   It is an objective of this invention to isolate, clone, and sequence further *eag* and *elk* genes and to use the nucleic acid sequences obtained to deduce the amino acid sequence of the proteins encoded by further *eag* and *elk* genes.

         It is another objective of this invention to isolate, 15   clone, and sequence other genes in the *eag*-like family and to use the nucleic acid sequences obtained to deduce the sequence of the protein encoded by other *eag*-like genes.

         It is another objective to determine the biophysical and pharmacological properties of the channels encoded.

20   It is a further objective to determine the distribution pattern of the mRNA of all known *eag*-like genes in sympathetic ganglia and sciatic nerve.

SUMMARY OF THE INVENTION

         Three new members of the mammalian *eag*-like gene 25   family are identified using rat sympathetic ganglia tissue.

         The *elk*1 gene has been isolated, cloned and the complete coding sequence (SEQ ID NO:1, Fig. 3) identified. The gene has been expressed in *Xenopus* oocytes and the deduced amino acid sequence (SEQ ID NO:2, Fig 2) of the elk1 protein

was also determined. The biophysical and pharmacological properties of the *elk*1 encoded channel has also been analyzed. The *elk2* gene was also isolated, cloned and sequenced (SEQ ID NO:13) and the amino acid sequence (SEQ ID NO:3) of the

5   protein deduced.

Further, another member of the *eag* family, the *eag2* gene was isolated, cloned and sequenced (SEQ ID NO:14) and its deduced amino acid sequence (SEQ ID NO:4) determined.

In addition, the mRNA distribution pattern in rat

10 sympathetic ganglia and sciatic nerve of all known *eag* genes was obtained using three sympathetic ganglia: one paravertebral ganglia, the superior cervical ganglia (SCG); and two prevertebral ganglia, the coeliac ganglia (CG) and the superior mesenteric ganglia (SMG).

15   The results indicate that The *elk*1 gene is expressed in both prevertebral and paravertebral ganglia at approximately equal levels. In contrast, the *elk2* gene is not expressed at detectable levels in sympathetic ganglia, although it is robustly expressed in the brain. Both *eag*

20 genes are expressed in sympathetic ganglia. The *eag*1 gene is expressed at high levels in all three sympathetic ganglia. The *eag2* gene is only expressed at significant levels in the prevertebral ganglia, the CG and SMG.

In addition to neurons, sympathetic ganglia also

25 contain glial cells (known as satellite cells and developmentally related to Schwann cells). To determine whether gene expression in ganglia could be due to expression in glial cells, *EAG* gene expression was examined in sciatic nerve, which contains a relatively pure population of Schwann

30 cells. Two of the seven *EAG* genes tested , *elk1* and *erg1,* are expressed in sciatic nerve (Fig. 4B).

A summary of *EAG* gene expression in sympathetic ganglia and sciatic nerve is shown in Table 1. At least six *EAG* genes are expressed in the sympathetic ganglia and all seven *EAG* genes are expressed in at least one neuronal tissue.

5   Two genes, *eag2* and *erg2*, are differentially expressed in sympathetic ganglia. Both genes are expressed at higher levels in prevertebral ganglia than paravertebral ganglia. The housekeeping gene cyclophilin was expressed at significantly lower levels in sciatic nerve RNA than in

10 ganglia or brain RNA (Fig. 4B). It is possible, therefore, that mRNA levels, as a fraction of total RNA, are significantly lower in sciatic nerve than in ganglia or brain. For this reason, the relative level of *elk1* and *erg1* expression in sciatic nerve described in Table 1 may be an

15 underestimate in comparisons between sciatic nerve and ganglia or brain.

Utilizing the information obtained from rat *elk1*, *elk2* and *eag2* the corresponding human genes can be obtained and the protein encoded thereby expressed.

20 The present invention thus provides isolated nucleotides from rat and human tissue encoding a polypeptide forming a potassium channel. The polypeptide is selected from the group consisting of elk1, elk2 and eag2. The elk1 polypeptide forms a channel having the characteristic of a

25 slowly activated outward rectifier, with an activation constant of about 100 ms at 0 mV with a threshold of -40 mV. The eag-like K channels also exhibits no apparent inactivation during de-polarizing voltage steps of up to several seconds.

The elk1 channel is characterized by having a

30 activation time constant of 676 ± 37 ms at 0 mV with a threshold of activation at -40 mV and a deactivation rate of

111 ± 7 ms at -50 mV at about pH 7. Based on our
observations, the activation/deactivation rate for elk1 is
dependent on the pH of the solvent. However, for elk2 and
eag2, the activation/deactivation rate is not as pH dependent.

5   The polypeptide elk1 has a molecular weight of between
100 kDa to 150 kDa, about 123 kDa. The polypeptides elk2 and
eag2 have a molecular weight similar to that of elk1 of
between 100 kDa to 150 kDa.

          The nucleic acids encoding elk1, elk2, or eag2 are
10   amplified by primers that selectively hybridize under
stringent conditions to sequences that hybridize to the
primers. For *elk1*, the primers are SEQ ID NO:5 and SEQ ID
NO:6: for *elk2*, the primers are SEQ ID NOS:9 and 10 and for
eag2, the primers are SEQ ID NOS:5 and 6. To obtain the
15   equivalent human *elk1, elk2* or *eag2*, the primers may be ·
selected by a comparison of the rat *elk1, elk2* or *eag2* nucleic
acid sequences respectively with the human EST sequences
listed in Genbank using the BLAST or PILEUP programs.
Sequences from the list of human EST which shows the highest
20   homology with a region of each of the rat sequences for *elk1*
elk2 or *eag2* are obtained. From the EST sequences obtained,
nucleotides of between 17 to 25 base pairs with at least 90%
homology between the rat and human sequences are selected for
use as primers.

25   In another aspect, the present invention provides an
antibody that selectively binds to the polypeptide elk1 elk2
or eag2 described above.

          The present invention further provides expression
factors comprising the nucleic acid sequences encoding *elk1,*
30   *elk2* or *eag2.*

The present invention further provides host cells transfected with the expression vectors described above.

In another aspect, the present invention provides a method of drug screening to ensure that the compounds identified for use as drugs for other diseases do not have a deleterious effect on the function of the elk1, elk2 or eag2 potassium channels. The method comprises the steps of: (a) contacting the compound with a eukaryotic host cell or cell membrane in which has been expressed a polypeptide comprising an alpha subunit of a potassium channel selected from the group consisting of elk1, elk2 and eag2, the potassium channel having the characteristic of a slowly activated outward rectifier; and (b) determining the functional effect of the compound upon the cell or cell membrane expressing the potassium channel. One example of the cell or cell membrane expressing the potassium channels are *Xenopus* oocytes or the membranes thereof.

In one embodiment, the functional effect is determined by measuring changes in current or voltage. In another embodiment, the polypeptide monomer polypeptide is recombinant. In another embodiment, the potassium channel is homomeric or heteromeric.

In another embodiment, the present invention provides a method of detecting the presence of elk1, elk2 or eag2 in mammalian tissue, the method comprising the steps of: (i) isolating a biological sample; (ii) contacting the biological sample with a reagent that selectively associates with elk1, elk2 or eag2; and, (iii) detecting the level of reagent that selectively associates with the sample. The reagent is selected from the group consisting of: antibodies specific for elk1, elk2 or eag2, oligonucleotide primers specific for *elk1,*

elk2 or eag2, and for *elk1, elk2 or eag2* nucleic acid probes. The mammalian tissue may be from rat or human.

The present invention also provides, in a computer system, a method of screening for mutations of *elk1, elk2* or *eag2* genes, the method comprising the steps of: (i) entering into the computer system a first nucleic acid sequence encoding an slowly activated outward rectifier potassium channel protein having a nucleic acid sequence of SEQ ID No:1, or comprising a nucleic acid sequence SEQ ID No:13 or SEQ ID No:14; and conservatively modified versions thereof; (ii) comparing the first nucleic acid sequence with a second nucleic acid sequence having substantial identity to the first nucleic acid sequence; and (iii) identifying nucleotide differences between the first and second nucleic acid sequences.

The second nucleic acid sequence may be associated with a disease state. Or, the computer readable substrate comprises the first and the second nucleic acid sequences.

The present invention further provides, in a computer system, a method for identifying a three-dimensional structure of elk1, elk2 or EAG2 polypeptides, the method comprising the steps of: (i) entering into the computer system an amino acid sequence of at least 10 amino acids of elk1, elk2 or eag2 or at least 30 nucleotides of a gene encoding the elk1, elk2 or eag2 polypeptide, the polypeptide having an amino acid sequence of SEQ ID No: 2, or comprising the partial amino acid sequence SEQ ID No:3 or SEQ ID No:4 and conservatively modified versions thereof; and (ii) generating a three-dimensional structure of the polypeptide encoded by the amino acid sequence.

In one embodiment, the amino acid sequence is a

primary structure and wherein said generating step includes
the steps of: (i) forming a secondary structure from said
primary structure using energy terms determined by the primary
structure; and (ii) forming a tertiary structure from said

5   secondary structure using energy terms determined by said
secondary structure. In another embodiment, the generating
step includes the step of forming a quaternary structure from
said tertiary structure using anisotrophic terms determined by
the tertiary structure. In another embodiment, the methods

10   further comprises the step of identifying regions of the
three-dimensional structure of the protein that bind to
ligands and using the regions to identify ligands that bind to
the protein. In another embodiment, a computer readable
substrate comprises the three dimensional structure of the

15   polypeptide monomer.

                    BRIEF DESCRIPTION OF THE DRAWINGS

        Fig. 1 is a schematic representation of the
classification of the K channel genes.

        Fig. 2 is an alignment of the *elk1, erg1* and *eag1*

20   deduced amino acid sequences. Identical residues are double
underlined and similar residues are single underlined. The
six hydrophobic domains (S1-6), and the pore (P) and the
putative cyclic nucleotide binding domain (cNBD) are
overlined.

25   Fig. 3 is the cDNA sequence of the *elk1* gene (SEQ ID
NO.1).

        Figs. 4A-4H show the electrophysiological
characteristics of the rat *elk1* expressed in *Xenopus* oocytes.

        Fig. 4A shows the voltage-clamp recordings of *elk1*

30   currents with voltage steps over the range of -60 to +50 mV

from a holding potential of -70 mV. Tail currents were
recorded at -50 mV.

Fig. 4B show the normalized conductance-voltage curve
of the elk1 channel. Conductance of the channel was
5 calculated as the current measured at the end of a 2 second
depolarizing step divided by the driving force (EK = -75 mV).
Data points are averages from 7 oocytes and were fitted with
the equation:

$$G/Gmax = 1/(1 + exp((V-Vn)/kn))$$

10 where Vn, the midpoint for activation, was 9.3 ± 0.4 mV, and
kn, the slope factor, was -13.1 ± 0.3 mV. Error bars are
s.e.m. and are not as large as the graph symbols.

Fig. 4C depicts the activation of elk1 and rat eag1
channels from different holding potentials. Holding
15 potentials were varied over the range -120 mV to -40 mV in 20
mV increments. Test potential was 0 mV. The difference in
time scale for the two types of channel is to be noted.

Fig. 4D shows the deactivation of elk1 channel tail
currents. Currents were activated by a 2500 ms step to +10 mV
20 followed by steps to potentials over the range -90 to -30 mV
in 10 mV increments. Time constants for deactivation are
indicated next to the appropriate trace. The trace at -70 mV
is not shown due to the small amplitude of the tail current.

Fig. 4E shows the activation of the elk1 channel in
25 control perfusion solution (pH = 7.7, left) and in perfusion
solution with pH lowered to 6.6 (right). Recordings show
current responses to voltage steps from a holding potential of
-70 mV. Test potentials are indicated next to selected
current traces.

30 Fig. 4F depicts conductance-voltage curves in
perfusion solutions with various pH values. Data points were

fitted with the equation:

$$G = Gmax/(1 + exp((V-Vn)/kn))$$

where the midpoint for activation Vn = 9.6, 8.6, 15.7, 25.2,
and 9.0 mV, the slope factor kn = -13.8, -13.7, -12.5, -12.3,
-13.4 mV and the maximum conductance Gmax = 26.8, 26.3, 24.1,
21.8, 25.8 μS for pH = 7.7, 8.6, 7.1, 6.6, and wash (7.7)
respectively.

Fig. 4G shows that E4031 does not block the elk1
channel. Voltage-clamp protocol is the same as in Fig. 4A.

Fig. 4H shows the elk1 channel is blocked by 1 mM
Ba2+. Voltage-clamp protocol is the same as in Fig. 4A.

Fig. 5A and 5B show autoradiographs of RNase
protection assays showing *EAG* potassium channel mRNA
expression in sympathetic ganglia, brain and sciatic nerve
determined by RNase protection analysis.

Fig. 5A shows samples tested: superior cervical
ganglia (SCG), coeliac ganglia (CG), superior mesenteric
ganglia (SMG) and brain. For the sympathetic ganglia, the
sample always contained 5 μg of total RNA. A variable amount
of total RNA was included in the brain samples as noted. In
gel a) *elk1* mRNA was expressed in all three sympathetic
ganglia. The brain sample contained 5 μg of total RNA. In gel
b) *elk2* mRNA was not expressed in sympathetic ganglia but was
moderately abundant in brain. The brain sample contained 2.5
μg of total RNA. In gel c) *eag1* mRNA was expressed at equal
levels in all three ganglia and was also expressed in brain.
The brain sample contained 14 μg of total RNA. In gel d) *eag2*
mRNA was barely detectable in the SCG but was expressed in the
two prevertebral ganglia, the CG and SMG, as well as in brain.
The brain sample contained 14 μg of total RNA.

- 14 -

Fig. 5B shows *elk1* and *erg1* mRNA expression in sciatic nerve (SN). Superior cervical ganglia (SCG) RNA served as a gpositive control. All samples contained 5 µg of total RNA.

The cyclophilin gene (cyc) was used as an internal positive control in all experiments to check for sample loss and equal loading.

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DETAILED DESCRIPTION OF THE INVENTION

INTRODUCTION

The present invention provides for the first time
three nucleic acids encoding respectively *elk1*, *elk2* or *eag2*
identified and cloned from rat and human tissue. The

polypeptides encoded by *elk1*, *elk2* or *eag2* are members of the voltage-gated eag-like family of potassium channel units (elk). Members of this family are polypeptides that are alpha subunits of slowly activated outward rectifier potassium

5   channels. Outward rectifier potassium channels have significant roles in maintaining the resting potential and in controlling excitability of a cell.

The invention also provides methods of screening for modulators, activators, and inhibitors of inward rectifier

10   potassium channels that contain a subunit selected from the group consisting of elk1, elk2 and eag2. Such modulators of outward rectifier channel activity are useful for treatment of hypertension, acute renal failure, chronic renal failure, diabetes insipidus, diabetic nephropathy, hypothyroidism,

15   hyperthyroidism, goiter, hypoparathyroidism, hyperparathyroidism, paacreatic insufficiency, diabetes, cystic fibrosis, sialorrhea, and salivary insufficiency.

Furthermore, the invention provides assays for elk1, elk2 or eag2 activity where for elk1, elk2 or eag2 acts as a

20   direct or indirect reporter molecule. Such uses of for elk1, elk2 or eag2 as a reporter molecule in assay and detection systems have broad applications, e.g., elk1, elk2 or eag2 can be used as a reporter molecule to measure changes in potassium concentration, in vitro, in vivo, and ex vivo, membrane

25   potential, current flow, ion flux, transcription, signal transduction, receptor-ligand interactions, second messenger concentrations. In one embodiment, elk1, elk2 or eag2 can be used as an indicator of current flow in a particular direction (outward potassium flow), and in another embodiment, elk1,

30   elk2 or eag2 can be used as an indirect reporter via attachment to a second reporter molecule such as green fluorescent protein. The invention provides for methods of

detecting elk1, elk2 or eag2 nucleic acid and protein
expression allows investigation of channel diversity provided
by elk1, elk2 or eag2 and the regulation/modulation of
heteromeric channel activity provided by elk1, elk2 or eag2 ,

5   as well as disease diagnosis for conditions such as
hypertension, acute renal failure, chronic renal failure,
diabetes insipidus, diabetic nephropathy, hypothyroidism,
hyperthyroidism, goiter, hypoparathyroidism,
hyperparathyroidism, pancreatic insufficiency, diabetes,

10   cystic fibrosis, sialorrhea, and salivary insufficiency
Finally, the invention provides for a method of screening for
mutations of elk1, elk2 or eag2 genes or proteins. The
invention includes, but is not limited to, methods of
screening for mutations in elk1, elk2 or eag2 with the use of

15   a computer. Similarly, the invention provides for methods of
identifying the three-dimensional structure of elk1, elk2 or
eag2 , as well as the resulting computer readable images or
data that comprise the three dimensional structure of elk1,
elk2 or eag2. Other methods for screening for mutations of

20   *elk*1, *elk*2 or *eag*2 genes or proteins include high density
oligonucleotide arrays, PCR, immunoassays and the like.
Functionally, each of the three elk1, elk2 or eag2 polypeptide
is an alpha subunit of an slowly activated outward rectifier
potassium channel. Such outward rectifier channels are

25   heteromeric or homomeric and contain typically contain four
alpha subunits or monomers each with two transmembrane
domains. Outward rectifier possium channels comprising elk1,
elk2 or eag2 can be heteromeric and may contain one or more
alpha subunits of elk1, elk2 or eag2 along with one or more

30   other alpha subunits from the EAG-like family. Each of elk1,
elk2 or eag2 can also comprise a homomeric slowly activated
outward rectifier channel. The presence of elk1, elk2 or eag2

in an slowly activated outward rectifier potassium channel
modulates the activity of the heteromeric channel and thus
enhances channel diversity.

5   Structurally, the nucleotide sequence of elk1 (SEQ ID
No:1) encodes a polypeptide of approximately 1102 amino acids
with a predicted molecular weight of approximately 123 kDa
(SEQ ID No:2) and a predicted range of 100-150 kDa.

          Specific regions of the elk1 nucleotide and amino acid
sequence may be used to identify polymorphic variants,
10   homologues, and alleles of elk1. This identification can be
made in vitro, e.g., under stringent hybridization conditions
and sequencing, or by using the sequence information in a
computer system for comparison with other nucleotide
sequences. Sequence comparison can be performed using any of
15   the sequence comparison algorithms discussed below.
Antibodies that bind specifically to elk1 can also be used to
identify alleles and polymorphic variants.

          Polymorphic variants and alleles of elk1 can be
confirmed by coexpressing the putative elk1 polypeptide and
20   examining whether it forms a heteromeric slow activated
outward rectifier potassium channel, when co-expressed with
another member of the EAG-like family such as elk2. This
assay is used to demonstrate that a protein having about 80%
or greater, preferably 90% or greater amino acid identity to
25   elk1 shares the same functional characteristics as elk1 and is
therefore a species of elk1. Typically, elk1 having the amino
acid sequence of SEQ ID No:2 is used as a positive control in
comparison to the putative elk 1 protein to demonstrate the
identification of a polymorphic variant or allele of elk1.

30   Similarly, polymorphic variants or alleles of elk2 or
eag2 may be identified. For elk2, the partial amino acid

sequence SEQ ID NO:3 may be used. For EAG2, the partial amino
acid sequence SEQ ID NO:4 may be employed.

elk1 nucleotide and amino acid sequence information
may also be used to construct models of a heteromeric slowly
5   activated outward rectifier potassium channels in a computer
system. These models are subsequently used to identify
compounds that can activate or inhibit heteromeric slowly
activated outward rectifier potassium channels comprising
elk1. Such compounds that modulate the activity of channels
10 comprising elk1 can be used to investigate the role of elk1 in
modulation of channel activity and in channel diversity.

The partial amino acid sequences for elk2 and eag2 may
also be used in like manner.

The isolation of biologically active elk1, elk2 and
15 eag2 for the first time provides a means for assaying for
inhibitors and activators of heteromeric slowly activated
outward rectifier potassium channels that comprise elk1, elk2
or eag2 subunits. Biologically active elk1, elk2 or eag2 is
useful for testing modulators, inhibitors, and activators of
20 slowly activated outward rectifier potassium channels
comprising subunits of elk1, elk2 or eag2 and other eag-like
members using in vivo and in vitro expression that measure,
e.g., changes in voltage or current. Such activators and
inhibitors identified using a slowly activated outward
25 rectifier potassium channel comprising at least one elk1, elk2
or eag2 subunit can be used to further study slowly activated
outward rectification, channel kinetics and conductance
properties of heteromeric channels. Such activators and
inhibitors are useful as pharmaceutical agents for treating
30 disorders such as hypertension, acute renal failure, chronic
renal failure, diabetes insipidus, diabetic nephropathy,

hypothyroidism, hyperthyroidism, goiter, hypoparathyroidism,
hyperparathyroidism, pancreatic insufficiency, diabetes,
cystic fibrosis, sialorrhea, and salivary insufficiency.
Methods of detecting elk1, elk2 or eag2 and expression of
5   channels comprising elk1, elk2 or eag2 are also useful for
diagnostic applications. For example, chromosomal
localization of elk1, elk2 or eag2 can be used to identify
diseases caused by and associated with elk1, elk2 or eag2 .
Methods of detecting elk1, elk2 or eag2 are also useful for
10 examining the role of elk1, elk2 or eag2 in channel diversity
and modulation.ef channel activity.

Definitions

As used herein, the following terms have the meanings
ascribed to them unless :specified otherwise.

15   The phrase "outward rectifier" activity or "outward
rectification" refers to a characteristic of a potassium-
channel allows potassium outflux, with little potassium
influx. For example, potassium ions flow outward through such
channels when the membrane potential is negative to EK, while
20 at more positive membrane potentials, inward currents may be
diminished (see, e.g., Pessia et al., EMBO J. 15:2980-2987
(1996)) EK is the membrane potential for potassium, the
combination of the voltage potential and [K$^+$] potential at
which there is no net flow of potassium ion. This value (-98
25 mV) is also known as the "reversal potential" or the "Nernst"
potential for potassium. Typically, the channel is composed
of four subunits and the channel can be heteromeric or
homomeric. The characteristic of outward rectification can be
measured by a variety of techniques for measuring changes in
30 current flow and ion flux through a channel, e.g., by changing
the [K+] of the external solution and measuring the activation
potential of the channel current (see, e.g., U.S. Patent No.

5,670,335), by measuring current with patch clamp techniques under different conditions, and by measuring ion flux with radiolabeled tracers or voltage-sensitive dyes under different conditions.

5   A "beta subunit" is a polypeptide monomer that is an auxiliary subunit of a cation-chanel composed of alpha subunits; however, beta subunits alone cannot form a channel (see, e.g., U.S. Patent No. 5,776,734). Beta subunits are known, for example, to increase the number of channels by
10   helping the alpha subunits reach the cell surface, change activation kinetics, and change the sensitivity of natural ligands binding to the channels. Beta subunits can be outside of the pore region and associated with alpha subunits comprising the pore region. They can also contribute to the
15   external mouth of the pore region.

        "elk1" refers to a polypeptide that is a subunit of an slowly activated outward rectifier potassium channel and a member of the EAG family of potassium channels. "elk1" therefore refers to polymorphic variants, alleles, mutants,
20   and closely related interspecies variants that: (1) have amino acid sequence identity of at least 60% , preferably about 80-90%, to elk1; or (2) have a nucleic acid sequence that specifically hybridize under stringent hybridization conditions to a sequence of about 17-25 base pairs selected
25   from SEQ ID No:1 or a conservatively modified variant thereof; or (4) are amplified by primers that specifically hybridize under stringent hybridization conditions to the same sequence as a primer set consisting of SEQ ID No:5 and SEQ ID No:6.

        "elk2" therefore refers to polymorphic variants,
30   alleles, mutants, and closely related interspecies variants that: (1) have amino acid sequence identity of at least 60% ,

preferably about 80-90%, to elk2; or (2) have a nucleic acid
sequence that specifically hybridize under stringent
hybridization conditions to a sequence of about 17-25 base
pairs selected from from SEQ ID No:13 or a conservatively
5   modified variant thereof; or (4) are amplified by primers that
specifically hybridize under stringent hybridization
conditions to the same sequence as a primer set consisting of
SEQ ID No:9 and SEQ ID No:10.

        "eag2" therefore refers to polymorphic variants,
10   alleles, mutants, and closely related interspecies variants
that: (1) have amino acid sequence identity of 50-90% ,
preferably about 80-90%, to eag2; or (2) have a nucleic acid
sequence that specifically hybridize under stringent
hybridization conditions to a sequence of about 17-25 base
15   pairs selected from sequence SEQ ID No:14 or a conservatively
modified variant thereof; or (4) are amplified by primers that
specifically hybridize under stringent hybridization
conditions to the same sequence as a primer set consisting of
SEQ ID No:5 and SEQ ID No:6.

20   The phrase "functional effects" in the context of
assays for testing compounds that may affect "elk1", "elk2" or
"eag2" channels includes the determination of any parameter
that is indirectly or directly under the influence of the
"elk1", "elk2" or "eag2" channel. It includes changes in ion
25   flux, membrane potential, current flow, transcription, G-
protein binding, phosphorylation or de-phosphorylation, signal
transduction, receptor-ligand interactions, second messenger
concentrations (e.g., cAMP, IP3, or intracellular Ca2+), as
measured in vitro, in vivo, and ex vivo, and also includes
30   other physiologic effects such increases or decreases of
neurotransmitter or hormone release.

By "determining the functional effect" is meant assays
for a compound that increases or decreases a parameter that is
indirectly or directly under the influence of "elk1", "elk2"
or "eag2". Such functional effects can be measured by any

5   means known to those skilled in the art, e.g., patch clamping,
voltage-sensitive dyes, whole cell currents, radioisotope
efflux, inducible markers, oocyte or tissue culture cell
expression of elk1, elk2 or eag2; transcriptional activation;
ligand binding assays; voltage, membrane potential and

10   conductance changes; ion flux assays; changes in intracellular
second messengers such as cAMP and inositol triphosphate
(IP3); changes in intracellular calcium levels;
neurotransmitter release, and the like.

"Inhibitors," "activators," and "modulators" of the

15   eag-like slowly activated outward rectifying potassium
channels comprising elk1, elk2 or eag2 refer to modulator
molecules identified using in vitro and in vivo assays for
channel function. Inhibitors are compounds that decrease,
block, prevent, delay activation, inactivate, desensitize, or

20   down regulate the channel. Activators are compounds that
increase, open, activate, facilitate, enhance activation,
sensitize or up regulate channel activity. Modulators include
naturally occurring and synthetic ligands, antagonists,
agonists, small chemical molecules and the like. Assays for

25   inhibitors and activators include, e.g., coexpressing a elk1
subunit with another eag-like alpha subunit in cells or cell
membranes, applying putative modulator compounds, and then
measuring flux of ions through the eag-like channel and
determining the functional effect of the modulator as

30   described above.

"Biologically active" "elk1", "elk2" or "eag2" refers
to "elk1", "elk2" or "eag2" that comprises a potassium channel

having slowly activated outward rectifier activity tested as
described above. Typically, the potassium channel is
heteromeric and contains at least one, preferably two subunits
of elk1 or elk2 or eag2 and subunits of another eag-like

5   family member such as eag1.

        The terms "isolated" "purified" or "biologically pure"
refer to material that is substantially or essentially free
from components which normally accompany it as found in its
native state. Purity and homogeneity are typically determined

10   using analytical chemistry techniques such as polyacrylamide
gel electrophoresis or high performance liquid chromatography.
A protein that is the predominant species present in a
preparation is substantially purified. In particular, an
isolated *elk*1 nucleic acid is separated from open reading

15   frames that flank the *elk*1 gene and encode proteins other than
*elk*1. The term "purified" denotes that a nucleic acid or
protein gives rise to essentially one band in an
electrophoretic gel. Particularly, it means that the nucleic
acid or protein is at least 85% pure, more preferably at least

20   95% pure, and most preferably 99% pure.

        "Nucleic acid" refers to deoxyribonucleotides or
ribonucleotides and polymers thereof in either single- or
double-stranded form. The term encompasses nucleic acids
containing known nucleotide analogs or modified backbone

25   residues or linkages, which are synthetic, naturally
occurring, and non-naturally occurring, which have similar
binding properties as the reference nucleic acid, and which
are metabolized in a manner similar to the reference
nucleotides. Examples of such analogs include, without

30   limitation, phosphorothioates, phosphoramidates, methyl
phosphonates, chiral-methyl phosphonates, 2-O-methyl
ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid
sequence also implicitly encompasses conservatively modified
variants thereof (e.g., degenerate codon substitutions) and
complementary sequences, as well as the sequence explicitly
5   indicated. The term nucleic acid is used interchangeably with
gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

The terms "polypeptide," "peptide" and "protein" are
used interchangeably herein to refer to a polymer of amino
acid residues. The terms apply to amino acid polymers in
10   which one or more amino acid residue is an analog or mimetic
of a corresponding naturally occurring amino acid, as well as
to naturally occurring amino acid polymers. Polypeptides can
be modified, e.g., by the addition of carbohydrate residues to
form glycoproteins. The terms "polypeptide," "peptide" and
15   "protein" include glycoproteins, as well as nonglycoproteins.

The term "amino acid" refers to naturally occurring
and synthetic amino acids, as well as amino acid analogs and
amino acid mimetics that function in a manner similar to the
naturally occurring amino acids. Naturally occurring amino
20   acids are those encoded by the generic code. Synthetic amino
acids include naturally occurring amino acids that are
modified, e.g., hydroxyproline, carboxyglutamate, and o-
phosphoserine. Amino acid analogs refers to compounds that
have the same basic chemical structure as a naturally
25   occurring amino acid, i.e., an carbon that is bound to a
hydrogen, a carboxyl group, an amino group, and an R group.,
e.g., homoserine, norTeucine, methionine sulfoxide, and
methionine methyl sulfonium. Such analogs have modified R
groups (e.g., norleucine) or modified peptide backbones, but
30   retain the same basic chemical structure as a naturally
occurring amino acid. Amino acid mimetics refers to chemical
compounds that have a structure that is different from the

general chemical structure of an amino acid, but that functions in a manner similar o a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides may be referred to by their commonly acceptd single-letter codes (A, T, G, C, U, etc.).

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Obtsuka et al., J. Biol. Chem. 260:260S-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon in an amino acid herein, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of

skill will recognize that each codon in a nucleic acid (except
AUG, which is ordinarily the only codon for methionine, and
TGG, which is ordinarily the only codon for tryptophan) can be
modified to yield a functionally identical molecule.
5   Accordingly, each silent variation of a nucleic acid which
encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will
recognize that individual substitutions, deletions or
additions to a nucleic acid, peptide, polypeptide, or protein
10 sequence which alters, adds or deletes a single amino acid or
a small percentage of amino acids in the encoded sequence is a
"conservatively modified variant" where the alteration results
in the substitution of an amino acid with a chemically similar
amino acid. Conservative substitution tables providing
15 functionally similar amino acids are well known in the art.
Such conservatively modified variants are in addition to and
do not exclude polymorphc variants and alleles of the
invention.

The following groups each contain amino acids that are
20 conservative substitutions for one another:

1)   Alanine (A), Glycine (G);
2)   Serine (S), Threonine (T);
3)   Aspartic acid (D), Glutamic acid (E);
4)   Asparagine (N), Glutamine (Q);
25   5)   Cysteine (C), Methionine (M);
6)   Arginine (R), Lysine (K), Histidine (H);
7)   Isoleucine (I), Leucine (L), Valine (V); and
8)   Phenylalanine (F), Tyrosine (Y), Tryptophan
(W).
30 (see, e.g., Creighton, Proteins (1984) for a discussion of
amino acid properties). .

Macromolecular structures such as polypeptide
structures can be described in terms of various levels of
organization. For a general discussion of this organization,
see, e g., Alberts et al., Molecular Biology of the Cell (3rd
5   ed., (1994) and Cantor & Schimmel, Biophysical Chemistry Part
I: The Conformation of Biological Macromolecules (1980).
"Primary structure" refers to the amino acid sequence of a
particular peptide. "Secondary structure" refers to locally
ordered, three dimensional structures within a polypeptide.
10   These structures are commonly known as domains. Domains are
portions of a polypeptide that form a compact unit of the
polypeptide and are typically 50 to 350 amino acids long.
Typical domains are made up of sections of lesser organization
such as stretches of ß-sheet and α-helices. "Tertiary
15   structure" refers to the complete three dimensional structure
of a polypeptide monomer. "Quaternary structure" refers to
the three dimensional structure formed by the non-covalent
association of independent tertiary units

A "label" is a composition detectable by
20   spectroscopic, photochemical, biochemical, immunochemical, or
chemical means. For example, useful labels include ^{32}P,
fluorescent dyes, electron-dense reagents, enzymes (e.g., as
commonly used in an ELISA), biotin, digoxigenin, or haptens
and proteins for which antisera or monoclonal antibodies are
25   available (e.g., the polypeptide of SEQ ID No:1 can be made
detectable, e.g., by incorporating a radiolabel into the
peptide, and used to detect antibodies specifically reactive
with the peptide).

As used herein a "nucleic acid probe or
30   oligonucleotide" is defined as a nucleic acid capable of
binding to a target nucleic acid of complementary sequence
through one or more types of chemical bonds, usually through

complementary base pairing, usually through hydrogen bond
formation. As used herein, a probe may include natural (i.e.,
A, G, C, or T) or modified bases (7-deazaguanosine, inosine,
etc.). In addition, the bases in a probe may be joined by a

5   linkage other than a phosphodiester bond, so long as it does
not interfere with hybridization. Thus, for example, probes
may be peptide nucleic acids in which the constituent bases
are joined by peptide bonds rather than phosphodiester
linkages. It will be understood by one of skill in the art

10 that probes may bind target sequences lacking complete
complementarily with the probe sequence depending upon the
stringency of the hybridization conditions. The probes are
preferably directly labeled as with isotopes, chromophores,
lumiphores, chromogens, or indirectly labeled such as with

15 biotin to which a streptavidin complex may later bind. By
assaying for the presence or absence of the probe, one can
detect the presence or absence of the select sequence or
subsequence.

    A "labeled nucleic acid probe or oligonucleotide" is

20 one that is bound, either covalently, through a linker or a
chemical bond, or noncovalently, through ionic, van der Waals,
electrostatic, or hydrogen bonds to a label such that the
presence of the probe may be detected by detecting the
presence of the label bound to the probe.

25   The term "recombinant" when used with reference, e.g.,
to a cell, or nucleic acid, protein or vector, indicates that
the cell, nucleic acid, protein or vector, has been modified
by the introduction of a heterologous nucleic acid or protein
or the alteration of a native nucleic acid or protein, or that

30 the cell is derived from a cell so modified. Thus, for
example, recombinant cells express genes that are not found
within the native (non recombinant) form of the cell or

express native genes that are otherwise abnormally expressed,
under expressed or not expressed at all.

A "promoter" is defined as an array of nucleic acid
control sequences that direct transcription of a nucleic acid.
5   As used herein, a promoter includes necessary nucleic acid
sequences near the start site of transcription, such as, in
the case of a polymerase II type promoter, a TATA element. A
promoter also optionally includes distal enhancer or repressor
elements, which can be located as much as several thousand
10   base pairs from the start site of transcription. A
"constitutive" promoter is a promoter that is active under
most environmental and developmental conditions. An
"inducible" promoter is a promoter that is active under
environmental or developmental regulation. The term "operably
15   linked" refers to a functional linkage between a nucleic acid
expression control sequence (such as a promoter, or array of
transcription factor binding sites) and a second nucleic acid
sequence, wherein the expression control sequence directs
transcription of the nucleic acid corresponding to the second
20   sequence.

The term "heterologous" when used with reference to
portions of a nucleic acid indicates that the nucleic acid
comprises two or more subsequences that are not found in the
same relationship to each other in nature. For instance, the
25   nucleic acid is typically recombinantly produced, having two
or more sequences from unrelated genes arranged to make a new
functional nucleic acid, e.g., a promoter from one source and
a coding region from another source. Similarly, a
heterologous protein indicates that the protein comprises two
30   or more subsequences that are not found in the same
relationship to each other in nature (e.g;, a, fusion
protein).

An "expression vector" is a nucleic acid construct,
generated recombinantly or synthetically, with a series of
specified nucleic-acid elements that permit transcription of a
particular nucleic acid in a host cell. The expression vector
5   can be part of a plasmid, virus, or nucleic acid fragment.
Typically, the expression vector includes a nucleic acid to be
transcribed operably linked to a promoter.

The terms "identical" or percent "identity," in the
context of two or more nucleic acids or polypeptide sequences,
10 refer to two or more sequences or subsequences (i.e., a human
*elk*1 monomer tail region) that are the same or have a
specified percentage of amino acid residues or nucleotides
(i.e., 80% identity, preferably 85%, 90%, or 95% identity)
that are the same, when compared and aligned for maximum
15 correspondence over a comparison window, as measured using one
of the following sequence comparison algorithms or by manual
alignment and visual inspection. Such sequences are then said
to be "substantially identical." This definition also refers
to the complement of a test sequence. Preferably, the percent
20 identity exists over a region of the sequence that is at least
about 25 amino acids in length, more preferably over a region
that is 50 or 100 amino acids in length.

For sequence comparison, one sequence acts as a
reference sequence, to which test sequences are compared.
25 When using a sequence comparison algorithm, test and reference
sequences are entered into a computer, subsequence coordinates
are designated, if necessary, and sequence algorithm program
parameters are designated. Default program parameters can be
used, as described herein, or alternative parameters can be
30 designated. The sequence comparison algorithm then calculates
the percent sequence identities for the test sequences
relative to the reference sequence, based on the program

parameters.

A "comparison window," as used herein, includes reference to a segment of contiguous positions having a length of at least about 25 nucleotides, usually about 50 to about

5   200 nucleotides, more usually about 100 to about 150 nucleotides in which a sequence may be compared to a reference sequence after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for

10   comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat 'l. Acad. Sci. USA 85:2444

15   (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, S75 Science Dr., Madison, WI), or by. manual alignment and visual inspection.

One example of a useful algorithm is PILEUP. PILEUP

20   creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the

25   progressive alignment method of Feng & Doolittle, J. Mol. Evol. 35:35 1-360 (1987). The method used is similar to the method described by Higgins & Sharp, CABIOS 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino-acids. The multiple

30   alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most

related sequence or cluster of aligned sequences. Two
clusters of sequences are aligned by a simple extension of the
pairwise alignment of two individual sequences. The final
alignment is achieved by a series of progressive, pairwise

5   alignments. The program is run by designating specific
sequences and their amino acid or nucleotide coordinates for
regions of sequence comparison and by designating the program
parameters. Using PILEUP, a reference sequence is compared to
other test sequences to determine the percent sequence

10   identity relationship using the following parameters: default
gap weight (3.00), default gap length weight (0.10), and
weighted end gaps. PILEUP can be obtained from the GCG
sequence analysis software package, e.g., version 7.0
(Devereaux et al., Nucleic Acids Res. 12:387-39S (1984)).

15   Another example of algorithm that is suitable for
determining percent .sequence identity and sequence similarity
is the BLAST algorithm, which is described in Altschul et al.,
J. Mol. Biol. 215:403-410 (1990). Software for performing
BLAST analyses is publicly available through the National

20   Center for Biotechnology Information
(http://www.ncti.nlm.nih.gov/). This algorithm involves first
identifying high scoring sequence pairs (HSPs) by identifying
short-words of length W in the query sequence, which either
match or satisfy some positive valued threshold score T when

25   aligned with a word of the same length in a database sequence.
T is referred to as the neighborhood word score threshold
(Altschul et al., supra). These initial neighborhood word
hits act as seeds for initiating searches to find longer HSPs
containing them. The word hits are extended in both

30   directions along each sequence for as far as the cumulative
alignment score can be increased. Extension of the word hits
in each direction are halted when: the cumulative alignment

score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The

5   BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10,

10 M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat 'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST

15 algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a

20 comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the

25 polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example,

30 where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two

molecules or their complements hybridize to each other under
stringent conditions, as described below. Yet another
indication that two nucleotide sequences are substantially
identical is that the same primers can be used to amplify both
5   sequences.

The phrase "selectively (or specifically) hybridizes
to" refers to the binding, duplexing, or hybridizing of a
molecule only to a particular nucleotide sequence under
stringent hybridization conditions when that sequence is
10   present in a complex mixture (e.g., total cellular or library
DNA or RNA).

The phrase "stringent hybridization conditions" refers
to conditions under which a probe will hybridize to its target
subsequence, typically in a complex mixture of nucleic acid,
15   but to no other sequences. Stringent conditions are sequence-
dependent and will be different in different circumstances.
Longer sequences hybridize specifically at higher
temperatures. An extensive guide to the hybridization of
nucleic acids is found in Tijssen, Techniques in Biochemistry
20   and Molecular Biology--Hybridization with Nucleic Probes,
"overview of principles of hybridization and the strategy of
nucleic acid assays" (1993). Generally, stringent conditions
are selected to be about 5-10°C lower than the thermal melting
point (Tm) for the specific sequence at a defined ionic
25   strength pH. The Tm is the temperature (under defined ionic
strength, pH, and nucleic concentration) at which 50% of the
probes complementary to the target hybridize to the target
sequence at equilibrium (as the target sequences are present
in excess, at Tm, 50% of the probes are occupied at
30   equilibrium). Stringent conditions will be those in which the
salt concentration is less than about 1.0 M sodium ion,
typically about 0.01 to 1.0 M sodium ion concentration (or

other salts) at pH 7.0 to 8.3 and the temperature is at least
about 30°C for short probes (e.g., 10 to 50 nucleotides) and at
least about 60°C for long probes (e.g., greater than 50
nucleotides). Stringent conditions may also be achieved with

5   the addition of destabilizing agents such as formamide. For
selective or specific hybridization, a positive signal is at
least two times background, preferably 10 times background
hybridization. Exemplary stringent hybridization conditions
can be as following: 50% formamide, 5X SSC, and 1% SDS,

10   incubating at 42°C, or, 5X SSC, 1% SDS, incubating at 65°C,
with a wash in 0.2X SSC, and 0.1% SDS at 65°C.

       Nucleic acids that do not hybridize to each other
under stringent conditions are still substantially identical
if the polypeptides which they encode are substantially

15   identical. This occurs, for example, when a copy of a nucleic
acid is created using the maximum codon degeneracy permitted
by the genetic code. In such cases, the nucleic acids
typically hybridize under moderately stringent hybridization
conditions. Exemplary "moderately stringent hybridization

20   conditions" include a hybridization in a buffer of 40%
formamide, 1 M NaCl, 1% SDS at.37°C, and a wash in 1X SSC at
45°C. A positive hybridization is at least twice background.
Those of ordinary skill will readily recognize that
alternative hybridization and wash conditions can be utilized

25   to provide conditions of similar stringency.

       "Antibody" refers to a polypeptide comprising a
framework region from an immunoglobulin gene or fragments
thereof that specifically binds and recognizes an antigen.
The recognized immunoglobulin genes include the kappa, lambda,

30   alpha, gamma, delta, epsilon, and mu constant region genes, as
well as the myriad immunoglobulin variable region genes.
Light chains are classified as either kappa or lambda. Heavy

chains are classified as gamma, mu, alpha, delta, or epsilon,
which in turn define the immunoglobulin classes, IgG, lgM,
IgA, IgD and IgE, respectively.

5   An exemplary immunoglobulin (antibody) structural unit
comprises a tetramer. Each tetrameter is composed of two
identical pairs of polypeptide chains, each pair having one
"light" (about 25 kDa) and one "heavy" chain (about 50-70
kDa). The N-terminus of each chain defines a variable region
of about 100 to 110 or more amino acids primarily responsible
10   for antigen recognition. The terms variable light chain (VL)
and variable heavy chain (VH) refer to these light and heavy
chains respectively.

       Antibodies exist, e.g., as intact immunoglobulins or
as a number of well characterized fragments produced by
15   digestion with various peptidases. Thus, for example, pepsin
digests an antibody below the disulfide linkages in the hinge
region to produce F(ab)': a dimer of Fab which itself is a
light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'2
may be reduced under mild conditions to break the disulfide
20   linkage in the hinge region, thereby converting the F(ab)'2
dimer into an Fab' monomer. The Fab' monomer is essentially
an Fab with part of the hinge region (see Fundamental
Immunology (Paul ed., 3d ed. 1993). While various antibody
fragments are defined in terms of the digestion of an intact
25   antibody, one of skill will appreciate that such fragments may
be synthesized de novo either chemically or by using
recombinant DNA methodology. Thus, the term antibody, as used
herein, also includes antibody fragments either produced by
the modification of whole antibodies, or those synthesized de
30   novo using recombinant DNA methodologies (e.g., single chain
Fv) or those identified using phage display libraries (see,
e.g., McCafferty et al., Nature 348:552554 (1990)).

An "anti-elk1 " antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by the *elk*1 gene, cDNA, or a subsequence thereof. Similarly, an anti-elk2 antibody is an antibody or antibody fragment that

5   specifically binds a polypeptide encoded by the *elk*2 gene, cDNA, or a subsequence thereof and an anti-eag2 antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by the *eag*2 gene, cDNA, or a subsequence thereof .

10   A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species,

15   or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen

20   specificity.

The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the

25   antigen.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the

30   protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the

specified antibodies bind to a particular protein at least two
times the background and do not substantially bind in a
significant amount to other proteins present in the sample.
Specific binding to an antibody under such conditions may

5   require an antibody that is selected for its specificity to a
particular protein. For example, polyclonal antibodies raised
to rat *elk*1 with the amino acid sequence of the tail region
encoded in SEQ ID No:2 can be selected to obtain only those
polyclonal antibodies that are specifically immunoreactive

10   with the tail region of rat elk1 and not with other proteins,
except for polymorphic variants and alleles of rat elk1. This
selection may be achieved by subtracting out antibodies that
cross react with molecules such as human elk1 and other elk
molecules such as elk2. A variety of immunoassay formats may

15   be used to select antibodies specifically immunoreactive with
a particular protein. For example, solid-phase ELISA
immunoassays are routinely used to select antibodies
specifically immunoreactive with a protein (see, e.g., Harlow
& Lane, <u>Antibodies, A Laboratory Manual</u> (1988), for a

20   description of immunoassay formats and conditions that can be
used to determine specific immunoreactivity). Typically a
specific or selective reaction will be at least twice
background signal or noise and more typically more than 10 to
100 times background.

25   The phrase "selectively associates with" refers to the
ability of a nucleic acid to "selectively hybridize" with
another as defined above, or the ability of an antibody to
selectively (or specifically) bind to a protein, as defined
above.

30   By "host cell" is meant a cell that contains an
expression vector and supports the replication or expression
of the expression vector. Host cells may be prokaryotic cells

such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells such as CHo, HeLa and the like.

"Biological sample" as used herein is a sample of biological tissue or fluid that contains *elk*1 or nucleic acid encoding elk1 protein. Such samples include, but are not limited to, tissue isolated from humans. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. A biological sample is typically obtained from a eukaryotic organism, preferably eukaryotes such as fungi, plants, insects, protozoa, birds, fish, reptiles, and preferably a mammal such as rat, mice, cow, dog, guinea pig, or rabbit, and most preferably a primate such as chimpanzees or humans.

Isolation of cDNA clones

According to the present invention, three rat *eag*-like genes, *elk*1 and *elk*2, and *eag*2 *were* isolated by PCR amplification of cDNAs from rat brain and SCG cDNA. For *elk*1 degenerate primers directed against the S1 and S5 regions were used. For *elk*2, the *elk*1 sequence was used to identify similar sequences in a GenBank search. Two overlapping expressed sequence tags were found to encode a closely related channel (GenBank accession numbers R35526 and R73353). These fragments encompassed part of the cytoplasmic amino terminal domain through to the S2 region. The complementary oligonucleotide sequences derived from these sequences were used to amplify cDNA clones from rat brain and SCG cDNA.

Based *on* the sequences of the cDNAs obtained, the deduced amino acid sequence of *elk*1 (SEQ ID NO:2) and a partial amino acid sequence of *elk*2 (SEQ ID NO:3) were determined.

Using the same primers for isolating the partial *elk*1

cDNA, the *eag2* gene was initially identified by PCR
amplification from rat brain cDNA and a partial amino acid
sequence of *eag2* (SEQ ID NO:4) was determined.

5   RNA was prepared from sympathetic ganglia and brain
using the *RNase* protection assay. Full length *elk1* and *eag1*
cRNA transcripts were synthesized *in vitro*. The full length
rat *elk1* cDNA was isolated as described. The full length rat
eag1 cDNA was amplified from rat SCG cDNA using the Expand
High Fidelity PCR system. Expression was conducted in *Xenopus*
10   oocytes. The details of the procedures used are described
hereinbelow.

          The human genes for *elk1*, *elk2* and *eag2* may be
obtained by searching the GenBank EST database using the
corresponding rat gene sequences, SEQ ID NO:1, SEQ ID NO:13 or
15   SEQ ID NO:14, either with the BLAST or PILEUP programs. The
search identifies EST from human tissues that are homologous
with the rat *elk1*, *elk2* or *eag2* gene. The region having a
sequence that is the most homologous with the rat *elk1* *elk2* or
eag2 gene is analyzed to select three sets of primers. In
20   accordance with the procedures described herein below, one of
the three primers is used to synthesize cDNA respectively for
human *elk1*, *elk2* and *eag2* gene from homogenized human fetal
brain, human adrenal and human stellate ganglia mRNA. The
entire open reading frame of the human *elk1* gene is then
25   determined using RACE PCR (Frohman, 1994) using the other two
EST specific primers as the initial anchor oliogonucleotides.
Several rounds RACE may be required to obtain the complete
open reading frame of the human *elk1*, *elk2* and *eag2*
respectively.

30   A systematic search for new members of the *EAG* gene
family was conducted using two different methods: i) novel

genes were isolated using degenerate primers combined with PCR amplification of cDNAs, ii) novel genes were identified in searches of GenBank and cDNAs then isolated by PCR. Three new genes were identified.

5 *elk*1

The *elk*1 gene was initially identified using the following degenerate primers directed against the S1 and S5 regions to PCR amplify partial cDNA clones from rat brain and SCG cDNA.

10   Forward: TTYAARRCNRYNTGGGAYTGG (SEQ ID NO:5)
        Reverse: RTACCADATRCANGCNAGCCARTG   (SEQ ID NO:6)

An initial sequence encompassing the entire open reading frame of the *elk*1 gene was determined by performing 5' and 3' RACE PCR (Frohman, 1994) using initial anchor
15 oligonucleotides complementary to the partial *elk*1 cDNA clone. Obtaining a sequence encompassing a complete open reading frame required several rounds of RACE in both directions using SCG cDNA as a template for amplification.

Once cDNAs were obtained that extended beyond both the
20 5' and 3' ends of the open reading frame, oligonucleotides complementary to non-coding regions at either end of the coding sequence were designed. Multiple full-length cDNA clones were amplified in independent PCR reactions from rat SCG cDNA using the Expand High Fidelity PCR system
25 (Boehringer-Mannheim, Indianapolis, IN). The following oligonucleotides were used to amplify full-length cDNA clones, giving a 52 b.p. and 381 b.p. 5' and 3' UTR respectively.

        forward: CGGGATCCTTGTGGACAAAC   (SEQ ID NO:7)

        reverse: TTCAGGAATGACAACCAGGC   (SEQ ID NO:8)

30   Two independent clones were sequenced, in their entirety, using a combination of manual and automatic

sequencing. Differences between the two sequences were
resolved by partial sequencing of a third independent full-
length cDNA clone. Sequence alignment of the deduced amino
acid sequence was performed using the ClustalW program
5   (Thompson et al, *Nucleic Acids Res.* (1994) 22:673-4680. The
cDNA sequence of the *elk1* gene is shown in Fig. 3.

elk2

The rat *elk2* gene was initially identified by
searching GenBank using the elk1 sequence. Two overlapping
10   expressed sequence tags were found to encode a closely related
channel (GenBank accession numbers R35526 and R73353). These
fragments encompassed part of the cytoplasmic amino terminal
domain through to the S2 region. The following complementary
oligonucleotide sequences derived from these sequences were
15   used to amplify cDNA clones from rat brain and SCG cDNA.

        forward: GTGATACCCATAAAGAATGAG   (SEQ ID NO:9)

        reverse: CGGAAATTCAGCACAATGTC   (SEQ ID NO:10)

The *elk2* sequence is
        AAGGGGGAGG TGGCCCTCTT CCTGGTCTCT CACAAGGACA TCAGTGAGAC
20   CAAGAACCGA GGAGGCCCTG ACAACTGGAA GGAGAGAGGT GGTGGCCGAC
        GCAGATATGG TCGGGCAGGA TCCAAAGGCT TTAATGCCAA TCGGAGGCGC
        AGCCGGGCGG TTCTCTACCA CCTCTCTGGT CACCTGCAGA AACAACCCAA
        GGGCAAGCAC AAACTCAATA AGGGTGTGTT TGGAGAGAAG CCAAATTTGC
        CCGAATATAA AGTCGCTGCT ATCCGGAAGT CACCCTTTAT CCTGCTGCAC
25   TGTGGGGCTC TGAGAGCCAC CTGGGATGGC TTCATCCTGC TCGCCACGCT
        CTACGTGGCT GTCACTGTGC CATACAGCGT GTGTGTGAGC ACAGCACGGG
        AGCCCAGTGC TGCCCGTGGC CCACCTAGTG TCTGTGACCT GGCCGTGGAA
        GTCCTCTTCA TCTT   (SEQ ID NO:13)
        The deduced amino acid sequence of elk2 is:

KGEVALFLVS HKDISETKNR GGPDNWKERG GGRRRYGRAG SKGFNANRRR

SRAVLYHLSG HLQKQPKGKH KLNKGVFGEK PNLPEYKVAA IRKSPFILLH

CGALRATWDG FILLATLYVA VTVPYSVCVS TAREPSAARG PPSVCDLAVE

VLFI   (SEQ ID NO:3)

5   The *elk*2 gene was clearly a member of a subfamily of
mammalian elk genes. The *elk*2 deduced amino acid sequence was
52% identical to *elk*1 and only 27% and 29% identical to rat
*erg*1 and *EAG*1 respectively over the region shown above.

       The rat *elk*2 gene was also used to search the GenBank
10   human EST to obtain the human *elk*2 gene, and elk2 polypeptide.

eag2

       The *eag2* gene was initially identified by PCR
amplification from rat brain cDNA using the same primers as
were used to isolate the partial rat elk1 cDNA. The reported
15   sequence encompasses a region between S1 and S5.

       The *eag2* sequence is:

GTGATTTTAA TTCTTACCTT CTACACCGCC ATCATGGTTC CTTACAACGT

TTCCTTCAAA ACAAAACAGA ACAATATCGC CTGGCTGGTT CTGGACAGCG

TGGTGGACGT TATTTTTCTG GTGGACATCG TTTTAAACTT TCACACGACT

20   TTTGTGGGGC CGGGTGGAGA GGTCATTTCT GACCCAAAAC TCATACGGAT

GAACTATCTG AAAACTTGGT TTGTGATTGA TCTGCTGTCT TGTTTACCTT

ATGACATCAT CAATGCCTTT GAAAATGTGG ATGAGGGAAT CAGCAGTCTC

TTCAGCTCTT TAAAGGTGGT ACGCCTCTTA CGCCTGGGCC GTGTTGCTAG

GAAACTGGAC CATTACCTGG AATATGGAGC AGCGGTCCTT GTGCTCCTGG

25   TATGTGTGTT TGGACTGGTT GCC   (SEQ ID NO:14)

       The deduced amino acid sequence of eag2 is:

VILILTFYTA IMVPYNVSFK TKQNNIAWLV LDSVVDVIFL VDIVLNFHTT

FVGPGGEVIS DPKLIRMNYL KTWFVIDLLS CLPYDIINAF ENVDEGISSL

FSSLKVVRLL RLGRVARKLD HYLEYGAAVL VLLVCVFGLV A

30   (SEQ ID NO:4)

The *eag2* gene was very similar to the previously identified rat *eag* gene (Ludwig et al., 1994), being 72% identical to rat *eag* (*eag1*) at the DNA sequence level over this region. The deduced amino acid sequence of eag2 was 92%

5   identical to eag1 over this region and only 42% and 45% identical to rat elk1 and erg1 respectively.

RNase Protection Assay

        The procedures for the preparation of RNA from sympathetic ganglia and brain and the performance of the RNase

10 protection assay were identical to those described previously. Dixon and McKinnon, <u>Eur. J. Neurosci.</u> (1996) 8:83-191.

        All RNA samples were prepared from pooled dissections of sympathetic ganglia from young adult Sprague-Dawley rats (4-5 weeks). The superior cervical, coeliac, and superior

15 mesenteric ganglia were used because they are relatively discrete and can be routinely identified for dissection. For RNA preparation three tissue samples were used: (i) the combined left and tight SCG, (ii) the right CG and (iii) the right SCG. Ganglia were carefully dissected free of host

20 tissue, rinsed in phosphate buffered saline and then frozen in liquid nitrogen. Frozen ganglia were then homogenized in guanidinium thiocyanate and total RNA was prepared by pelleting over a CsCl step gradient. All RNA smaples were carefully quantitated by spectrophotometic analysis and the

25 integrity of the RNA samples was confirmed by analysis on a denaturing agarose gel. Sciatic nerve RNA was prepared similarly following dissection of sciatic nerve from rat hind limb and rapid freezing in liquid N_2. RNA expression was quantitated directly from dried RNase protection gels using a

30 PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

        The RNase protection assay was performed as follows.

RNA samples were assayed as a set coming from the same group
of animals. The data were obtained from independent sets of
RNA samples. For each sample point 5 or 10 µg of total RNA
was used in the assay. For the positive control brain or

5   atria sample, 5 µg of total RNA was used. In experiments
where the aim was to compare the level of expression of two
different K channels, the RNA probes were made using two DNA
templates in the same reaction mixture to minimize differences
in the specific activity of the two probes, For comparisons

10   between probes, the data were corrected for differences in
specific activity of the probes by assuming that the specific
activity was directly proportional to the number of UTPs in
each sequence. A probe for the rat cyclophilin gene was
included in the hybridization as an internal control. The

15   cyclophilin probe had a specific activity approximately
sevenfold lower than that of the K channel probes. 10 µg of
yeast tRNA was used as a negative control to test for the
presence of probe self-protection bands. RNA expression was
quantitated directly from dried Rnase protection gels that

20   were placed in a β-counter (Betascope 603, Biogen). Specific
counts for each band were determined by subtracting a
background signal determined from the tRNA lane using a
counting rectangle of the same size and relative location as
was used for the sample band.

25   The following DNA templates were used for the RNase
protection assays.

           eag1: Encompasses nucleotides 855 to 1162 of rat eag
(Ludwig et al, EMBO J. (1994) 13:451-4458. GenBank Accession
number Z34264).

30   eag2: Used partial eag2 cDNA described above (GenBank
Accession number: AF073891).

elk1: Encompasses nucleotides 710 to 1177 of rat elk1 described above (GenBank Accession number: AF061957).

elk2: Used partial elk2 cDNA described above (GenBank Accession number: AF073892).

5   Expression in Xenopus Oocytes

Full-length rat elk1 and eag1 cRNA transcripts were synthesized in vitro. Isolation of the full-length rat elk1 cDNA is described above. The full-length rat eag1 cDNA was amplified from rat SCG cDNA using the Expand High Fidelity PCR

10 system (Boehringer-Mannheim, Indianapolis, IN). The following oligonucleotides were used to amplify full-length EAG1 cDNA clones:

        forward : TGCTGCGGTGAGACACG   (SEQ ID NO:11)

        reverse : TGGTCATGTGTTTGGTGCG   (SEQ ID NO:12)

15   Oocytes were prepared from mature female Xenopus laevis using established procedures (Colman, 1984, incorporated herein by reference). Frogs were anaesthetized in ice water containing a 0.1% solution of Tricaine. Defolliculation was performed by incubation for 2 hours in 2

20 mg/ml collagenase (Type VIII, Sigma, St. Louis, MO) in Ca^{2+}-free OR2 oocyte medium with gentle agitation. Oocytes were stored in OR3 solution (50% L-15 medium (GIBCO, Gaithersburg, MD), 1 mM glutamine, 15 mM Na-HEPES (pH 7.6), 0.1 mg/ml gentamicin) at 18°C. Oocytes were injected with 50 nl of cRNA

25 (~0.3 ng/nl) using a microdispenser and a micropipette with tip diameter of 10-20 µm. Injected oocytes were incubated at 18°C for 24-48 hours prior to analysis.

        Oocytes were voltage-clamped using a two-microelectrode voltage clamp. Intracellular electrodes filled

30 with 3 M KCl with resistances of 0.5-3 MΩ were used. The

standard extracellular recording solution contained: 80 mM
NaCl, 5 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, and 5 mM Na-HEPES (pH
7.6). Data collection and analysis were performed using
pClamp software (Axon Instruments, Foster City, CA). E4031 was
5   obtained from Eisai Co. Ltd. (Tokodai, Japan).

Human *elk1*

Two methods described below are useful for obtaining
the cDNAs that extend beyond the 5' and 3' ends of the open
reading frame of the human *elk1* gene.

10   Method 1

The DNA sequence for the rat *elk1* gene (SEQ ID NO:1)
is used to search the GenBank data base in order to identify
expressed sequence tags (EST) from human tissues that are
homologous with a part of the gene. The ESTs are not be known
15   to encode any part of the human *elk1* gene.

Using the *elk1* gene (SEQ ID NO:1) and applying the
BLAST program, a human EST with the highest degree of homology
to the *elk1* gene is identified.

Three primers of 17-25 base pairs having at least 90%
20   homology with a region of rat *elk1* are selected from the EST
sequence identified. The primer from the most 3' end of the
EST is used to make cDNA from RNA isolated from human tissue.
The tissue sources include, but are not restricted to, human
brain, human adrenal and human stellate ganglia. cDNA
25   synthesis is performed using established techniques as
described by the manufacturers of the reverse transcriptase
enzyme. The temperature recommended by the manufacturer and ,
a higher temperature (50°C) are used to synthesize the cDNA.
The higher temperature of 50°C is used to maximize the
30   specificity of the reaction for the human *elk1* gene.

The entire opening reading frame of the human elk1 gene is obtained using 5' RACE PCR (Frohman, 1994) using the two other primers specific for the EST as the initial anchor oligonucleotides. The procedure is described for the rat *elk1*
5   gene herein above. Obtaining a sequence encompassing a complete open reading frame may require several rounds of RACE.

The procedures for the preparation of RNA and the expression of the cDNA transcripts in *Xenopus Oocytes* are as
10 described above for rat *elk1*.

Method 2

Degenerate oligonucleotides are used to clone a partial cDNA sequence using human brain, adrenal or stellate cDNA as a template for amplification. Initially, the primers
15 selected as described above are directed against the core S1 to S6 region of the gene. However, other regions may also be used.

The primers selected include codons for amino acids that are specific for the *elk1* gene and not found in related
20 genes.

The entire opening reading frame of the human *elk1* gene is determined using 5' and 3' RACE PCR (Frohman, 1994) using initial anchor oligonucleotides complementary to the partial human elk1 cDNA clone.

25   Obtaining a sequence encompassing a complete open reading frame may require several rounds of RACE in both directions.

Once cDNAs are obtained that extend beyond both the 5' and 3' ends of the open reading frame, oligonucleotides are
30 selected complementary to non-coding regions at either end of

the coding sequence. Multiple full-length cDNA clones are amplified in independent PCR reactions from human brain, adrenal or stellate cDNA using the standard amplification techniques as described the manufacturer of the DNA

5   polymerase.

Independent clones are sequenced, in their entirety, using automatic sequencing. Sequence differences are resolved by partial sequencing of an independent cDNA clone. Sequence alignment of the deduced amino acid sequence are performed

10   using the ClustalW program (Thompson et al., 1994).

Results

Isolation of Full-length *elk*1 cDNA Clone

A systematic search for genes related to the *Drosophila elk* gene and the mammalian *eag* gene was conducted

15   and three new genes are identified in rat: *elk*1, *elk*2 and *eag*2 and in human (see Methods). Of these three genes the *elk*1 gene is of the most interest since no *elk* channel had been expressed and characterized previously. In addition the rat *elk*1 gene is expressed in sympathetic ganglia, a system that

20   has been well characterized both in terms of potassium channel function as well as gene expression (Dixon and McKinnon, 1996, supra; Shi *et al.*, 1997, supra).

The deduced amino acid sequence of the *elk*1 gene suggests that it encodes a protein 1,102 amino acid residues

25   in length with an estimated molecular weight of 123 kDa (Fig. 1). Assignment of the initiator methionine and the start of the open reading frame was facilitated by the high degree of similarity in this region between the *elk*1 and *Drosophila elk* sequences. There were multiple stop codons in all three

30   reading frames around the selected stop codon.

The deduced elk1 amino acid sequence was 41% identical to the *Drosophila elk* sequence. Identity with other members of the *EAG* gene family was significantly lower, ranging from 26 to 31%. This result suggests that the *elk1* gene is a

5 mammalian equivalent of the *Drosophila elk* gene. A similar level of identity between mammalian *erg* genes and their *Drosophila* counterpart has been found previously to be in the range 41 to 44%. Shi et al, 1997, supra. Identity between the rat *eag1* gene and the *Drosophila eag* gene is somewhat

10 higher, however, at 51%.

There is a high degree of similarity between the *elk*1 deduced amino acid sequence and the *erg* and *EAG* sequences in the amino terminus, the hydrophobic core and the putative cyclic nucleotide binding domain (Fig. 1). The *elk*1 sequence

15 shares the pore signature sequence GFG with both the *erg* and EAG channels, in contrast to most other potassium channels, which have GYG in the equivalent position (Wei et al, 1996, supra). The *elk*1 protein has a relatively large cytoplasmic carboxyl domain compared to the other two channels and there

20 is a distinctive run of negatively charged residues in this region, with 11 of 12 residues being either glutamate or aspartate. A similar but much shorter sequence is seen in *EAG*1 in the equivalent position.

25 Functional Expression of *elk*1 channels

It was of interest to compare the biophysical properties of the elk1 channel with those of eag and erg channels, since all three channels share significant regions of sequence identity. eag and erg channels have one common

30 feature, they are relatively slowly activating compared to Kv-class (*Shaker*-related) channels. This property is also shared

by the elk1 channel (Fig. 4A). The elk1 channel is one of the slowest activating EAG channels, having a time constant for activation of 676 ± 37 ms (n = 7) at 0 mV. The threshold for activation is around -40 mV and the conductance-voltage curve

5   can be fit with a simple Boltzmann distribution (Fig 4B: $V_n = 9.3 \pm 0.4$ mV and $k_n = -13.1 \pm 0.3$ mV (n = 7)).

In a simple activation protocol such as the one shown in Figure 3A, the elk1 channel has a similar waveform to eag channels: it is a slowly activating outward rectifier. he

10   elk1 channel exhibits no apparent inactivation during depolarizing voltage steps of up to several seconds, similar to the mammalian eag1 channel (Ludwig et al, 1994, supra; Robertson et al, 1996, supra). In contrast, erg channels have very fast inactivation rates, significantly faster than their

15   activation rates, which results in an apparent inward rectification at positive membrane potentials (Sanguinetti et al, 1995, supra; Spector et al, J. Gen. Physiol. (1996) 107:11-619).

One particularly striking feature of the eag channels

20   is that the rate of activation is strongly dependent upon the holding potential, activation is significantly faster from depolarized than hyperpolarized holding potentials. This suggests that during the activation process the eag channel undergoes a slow, voltage-dependent transition between at

25   least two distinct closed states before reaching the open state (Ludwig et al., 1994, supra; Robertson et al., 1996, supra). The elk1 channel shows no obvious change in the activation rate from different holding potentials, in marked contrast to the results for eag channels over a similar range

30   of holding potentials (Fig. 4C). In this respect, the elk1 channel is more similar to erg channels, which also do not exhibit a significant shift in activation rates with changes

in holding potential.

There are some other kinetic properties for which the elk1 channel is more similar to erg than eag channels. The rate of deactivation of the elk1 channel is relatively slow,
5   with a time constant of 111 ± 7 ms (n = 7) at -50 mV (Fig 4D). Deactivation rates for erg channels are also very slow, with deactivation time constants in the range of 100 ms up to several seconds at -50 mV (Sanguinetti et al, 1995, supra). In marked contrast, the deactivation rate of the mammalian
10   eag1 channel is very fast, with a time constant of 1-6 ms (Terlau et al, Pflugers Arch. (1996) 432:301-312).

Activation of the elk1 channel was strongly dependent on the pH of the external bath solution (Fig. 4E). Following acidification of the external perfusate solution, the
15   conductance-voltage curve was shifted significantly to more positive membrane potentials (Fig. 4F). The midpoint of the activation curve was shifted from 10.4 ± 0.4 mV at pH = 7.7, to 17.0 ± 0.7 mV at pH = 7.1 (p < 0.05), to 28.1 ± 1.5 mV at pH = 6.6 (p < 0.001). The slope factor was not significantly
20   affected by changes in external pH (p > 0.5). Changing the external solution to a more alkaline pH did not produce significant shifts in the activation-conductance curve (Fig. 4F). A somewhat similar dependence of activation on external pH is seen for mammalian eag channels (Terlau et al, 1996,
25   supra), although for eag channels there is also significant slowing of the activation kinetics by acidic pH, which is not seen for elk1 channels.

The elk1 channel was almost completely resistant to the application of two common potassium channel blockers, TEA
30   and 4-AP. Concentrations of up to 10 mM of either drug had limited or no effects on the channel. Another class of

potassium channel blockers, methanesulfonanilide drugs such as
E4031, are useful rEAGents because they are apparently quite
selective for cloned and native *erg* channels at concentrations
less than ~10 μM (Sanguinetti and Jurkiewicz, <u>J. Gen. Physiol.</u>

5   (1990) 96:95-215). These compounds have not been tested
systematically on closely related channels, however, and for
this reason we examined the effects of E4031 on the elk1
channel. E4031 had almost no effect at concentrations up to
10 μM (Fig. 4G). Barium ions also have some potential for

10   differentiating among the various *EAG*-family channels. The
elk1 channel is more sensitive than the *EAG1* channel to Ba^{2+}
ions, being 85% blocked by 1 mM Ba^{2+} in the external solution
(Fig. 4H). In comparison, the *EAG1* channel is blocked less
than 40% by 1 mM Ba^{2+} (see also Terlau et al., 1996, supra).

15   <u>*eag* Gene Expression in Sympathetic Ganglia</u>

To date the functional roles of eag, erg and elk
channels have been poorly characterized *in vivo*. One
potentially useful model system to study the function of these
channels is the peripheral sympathetic ganglia. For this

20   reason, we determined the distribution of *elk* and *eag* gene
expression in three sympathetic ganglia: one paravertebral
ganglia, the superior cervical ganglia (SCG) and two
prevertebral ganglia, the coeliac ganglia (CG) and the
superior mesenteric ganglia (SMG) (Fig. 5A). The *elk1* gene is

25   expressed in both prevertebral and paravertebral ganglia at
approximately equal levels. In contrast, the *elk2* gene is not
expressed at detectable levels in sympathetic ganglia,
although it is robustly expressed in the brain. Both *eag*
genes are expressed in sympathetic ganglia. The *eag1* gene is

30   expressed at high levels in all three sympathetic ganglia.
The *eag2* gene is only expressed at significant levels in the
prevertebral ganglia, the CG and SMG.

In addition to neurons, sympathetic ganglia also contain glial cells (known as satellite cells and developmentally related to Schwann cells). To determine whether gene expression in ganglia could be due to expression in glial cells we examined *eag* gene expression in sciatic nerve, which contains a relatively pure population of Schwann cells. Two of the seven *eag* genes tested, *elk*1 and *erg*1 are expressed in sciatic nerve (Fig. 5B).

A summary of *EAG* gene expression in sympathetic ganglia and sciatic nerve is shown in Table 1. At least six *EAG* genes are expressed in the sympathetic ganglia and all seven EAG genes are expressed in at least one neuronal tissue. Two genes, *eag*2 and *erg*2, are differentially expressed in sympathetic ganglia. Both genes are expressed at higher levels in prevertebral ganglia than paravertebral ganglia. The housekeeping gene cyclophilin was expressed at significantly lower levels in sciatic nerve RNA than in ganglia or brain RNA (Fig. 5B). It is possible, therefore, that mRNA levels, as a fraction of total RNA, are significantly lower in sciatic nerve than in ganglia or brain. For this reason, the relative level of *elk*1 and *erg*1 expression in sciatic nerve described in Table 1 may be an underestimate in comparisons between sciatic nerve and ganglia or brain.

It has been found in contrast to the *erg* genes, the *elk* genes are not expressed in heart tissue. This characteristic combined with the difference in electrophysiological characteristics should provide a tool for identifying pharmaceuticals which would block the K channels differentially. In this manner, K channel blockers which would intervene in arrythmia without interfering with the sympathetic nervous system may be identified.

57

Table 1

Distribution of EAG Potassium Channel Gene Expression
in Rat Sympathetic Ganglia, Brain and Sciatic Nerve

	SCG	CG	SMG	Brain	Sciatic Nerve
eag1	+++	+++	+++	++++	-
eag2	+/-	+	+	++	-
erg1	+++++	+++++	+++++	+++++	+++
erg2	+	++	+++	-	-
erg3	+++	+++	+++	++++	-
elk1	+++	+++	+++	+/-	++
elk2	-	-	-	+++	-

Key:
+++++ very high, ++++ high, +++ moderate, ++ low, + very low,
+/- just detectable, - negative

Semi-quantitative analysis of the relative levels of expression of all the EAG genes
was achieved by a combination of radioactive counting of individual gels and visual
comparison of autoradiographs from multiple experiments. In every case the data are based on
multiple independent experiments (n = 2 to 6). Data for sciatic nerve may be subject to a
systematic underestimate (see Results). Data for the erg gene expression in sympathetic
ganglia is adapted from Shi et al. (1997).

Discussion

The complete coding sequence of a new potassium channel gene, the rat *elk1* gene, and partial sequences for two other *eag* genes: *eag2* and *elk2* and the procedures for obtaining the human *elk1*, *eag2* and *elk2* have been provided. The *elk1* and *elk2* genes are mammalian equivalents of the *Drosophila elk* gene. When expressed in a heterologous expression system, the elk1 channel has some similar biophysical properties compared to the related eag and erg channels, but also has some clearly distinct properties. In particular, the elk1 channel activates very slowly and also deactivates slowly. The elk1 channel shows little or no inactivation during sustained depolarizing voltage steps.

Identification of three new *EAG* genes in addition to the previously known genes and the demonstration that all the *eag* genes are expressed in at least some region of the nervous system raises obvious questions about the function of EAG channels in the mammalian nervous system. At least six *EAG* genes are expressed in sympathetic ganglia, which is a large number of related genes to be expressed in a tissue that contains a relatively homogeneous population of cells. This result suggests that EAG channels are functionally important with, at most, only partially overlapping functions among the different family members. To date, however, there is little convincing data on the physiological function of any of these channels in the mammalian nervous system. In *Drosophila*, mutations in the *eag* and *erg* genes produce hyperexcitability, suggesting a role for these channels in regulating neuronal electrical activity. Ganetzky and Wu, J. Neurogenetics (1983) 1:17-28; Titus et al, J. Neurosci. (1997) 17:85-881; Wang et al, J. Neurosci. (1997) 17:82-890).

Very little information is currently available on the physiological role of elk channels or the related EAG channels in mammals. Both channels have relatively positive activation curves which limit their ability to affect sub-threshold

5   electrical excitability. They activate too slowly to affect the shape of the action potential and, even if they are neuronally expressed, it is difficult to understand how they might affect neuronal excitability. It is possible that heteromultimerization with ß subunits might modify the

10   functional properties of these channels but there is currently no direct evidence to support such a possibility. The *elk*1 gene is expressed in sciatic nerve, which suggests that elk1 channels may function in the glial cells of sympathetic ganglia rather than in neurons.

15   It has been suggested that EAG subunits co-assemble with more rapidly activating Kv channel subunits to produce heteromers with novel kinetic properties. The *Drosophila* eag channel appears to modify the inactivation properties of the Shaker B channel, possibly by co-assembly into a heteromeric

20   channel (Chen et al, Neuron (1996) 17:35-542). It has yet to be shown, however, that the mammalian Kv channels can be modified by co-assembly with mammalian eag subunits. A recent report of a current expressed in human neuroblastoma cells that has striking similarities to homomeric eag channels

25   expressed in heterologous expression systems (Meyer and Heinemann, J. Physiol. (1998) 508:9-56) suggests that mammalian eag subunits may assemble as independent channels rather than as components of heteromultimeric complexes.

         In conclusion, there are a large number of EAG

30   channels expressed in the mammalian nervous system and these channels have a wide diversity of biophysical properties. Surprisingly, the physiological function that any EAG channel

performs in the nervous system has yet to be determined. In
the past, the sympathetic nervous system has proven to be a
good model system in which to study potassium channels and a
large number of different potassium channel currents have been
5   identified (Brown et al, J. Auton. Nerv. Syst. (1982) 6:23-35;
Pennefather et al, Proc. Natl. Acad. Sci. (1985) 82:040-3044;
Belluzzi and Sacchi, , Prog. Biophys. Molec. Biol. (1991)
55:1-30; Wang and McKinnon, J. Physiol. (1995) 485:19-335).
There is, however, a clear excess in the number of potassium
10 channel genes expressed in sympathetic ganglia relative to the
number of physiologically identified potassium currents. It
will be technically difficult to further refine the
electrophysiological techniques necessary to resolve more
distinct potassium currents although single channel recordings
15 have provided anecdotal evidence for diversity within
apparently homogeneous macroscopic currents. These technical
limitations may make it necessary to use genetic approaches
such as gene knock-outs to establish the physiological
function of the EAG channels in the mammalian nervous system.

20   The present invention provides the gene sequences for
elk1, elk2 and eag2 to enable these genetic approaches for
further refining our understanding of the K channels and their
functions. The genetic and amino acid sequence information is
also useful for the development of treatment methods and
25 pharmaceuticals for the treatment of specific disease
conditions. Further the present invention provides methods by
which drug candidates can be screened to avoid averse effects
on the elk1, elk2 and eag2 potassium channels.

         Although the invention has been described with
30 specific embodiments and examples, they are intended to limit
the scope of the invention.

We claim:

1. An isolated nucleic acid encoding a polypeptide comprising an alpha subunit forming a eag-like potassium channel having the characteristic of slowly activated

5   outward rectification selected from the group consisting of *elk1*, *elk2* and *eag2*.

2. The isolated nucleic acid according to claim 1 encoding a polypeptide forming a potassium channel for which the activation time constant for outward rectification is

10 3. The isolated nucleic acid according to claim 2 wherein the activation time constant for outward rectification is at least 100 ms at 0 mV with a threshold activation at - 40 mV.

4. The isolated nucleic acid according to claim 2 wherein

15   the activation time constant for outward rectification is 676 ± 37 ms at 0 mV with a threshold activation at -40 mV at about pH 7.

5. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide having a molecular weight of

20   between 100 to 150 kDa.

6. The isolated nucleic acid of claim 1, wherein the polypeptide has a molecular weight of about 123 kDa.

7. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes human elk1.

25 8. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes rat elk1.

9. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes human elk2.

10. The isolated nucleic acid of claim 1, wherein the nucleic

30   acid encodes human eag2.

11. The isolated nucleic acid sequence of claim 7, wherein the nucleic acid has a nucletide sequence of SEQ ID No:1.

12. The isolated nucleic acid sequence of claim 8 wherein the
     nucleic acid is at least 80% homologous to SEQ ID NO:1.

13. The isolated nucleic acid of claim 7, wherein the nucleic
     acid is isolated from superior cervical ganglia, coeliac
5   ganglia, superior mesentreric ganglia, foetal brain,
     adrenal or stellate ganglia by PCR using primers that
     selectively hybridize under stringent hybridization
     conditions to a pair of primers:
     5' TTY AAR RCN RYN TGG GAY TGG 3'   (SEQ ID No:5) and
10   3' RTA CCA DAT RCA NGC NAG CCA RTG 5'   (SEQ ID No:6)
     and amplified using a pair of primers:
     5' CGG GAT CCT TGT GGA CAA AC 3'   (SEQ ID NO:7)
     3' TTC AGG AAT GAC AAC CAG GC 5'   (SEQ ID NO:8)..

14. The isolated nucleic acid of claim 8 encoding a
15   polypeptide and specifically hybridizes under stringent
     conditions to SEQ ID No:1.

15. The isolated nucleic acid of claim 8, wherein said
     nucleic acid selectively hybridizes under moderately
     stringent hybridization conditions to a nucleotide
20   sequence of SEQ ID No:1.

16. The isolated nucleic acid of claim 1, wherein said
     nucleic acid encodes the polypeptide SEQ ID NO:3.

17. The isolated nucleic acid of claim 1, wherein said
     nucleic acid comprises the partial sequence SEQ ID NO:13.

25 18. The isolated nucleic acid of claim 15, wherein the
     nucleic acid is isolated from superior cervical ganglia
     by PCR using primers that selectively hybridize under
     stringent hybridization conditions to a pair of primers:
     5' TTY AAR RCN RYN TGG GAY TGG 3'   (SEQ ID No:5) and
30   3' RTA CCA DAT RCA NGC NAG CCA RTG 5'   (SEQ ID No:6)
     and amplified with a pair of primers:

5' GTG ATA CCC ATA AAG AAT GAG 3' (SEQ ID NO:9)

3' CGG AAA TTC AGC ACA ATG TC 5'   (SEQ ID NO:10).

19. The isolated nucleic acid of claim 1, wherein said
     nucleic acid partially encodes the polypeptide SEQ ID
     NO:4.

20. The isolated nucleic acid of claim 1, wherein said
     nucleic acid comprises the partial sequence SEQ ID NO:14.

21. The isolated nucleic acid of claim 18, wherein the
     nucleic acid is isolated from brain tissue by PCR using
     primers that selectively hybridize under stringent
     hybridization conditions to a pair of primers:
     5' TTY AAR RCN RYN TGG GAY TGG 3' (SEQ ID No:5) and
     3' RTA CCA DAT RCA NGC NAG CCA RTG 5'   (SEQ ID No:6)
     and amplified by PCR using a pair of primers:
     5' CGG GAT CCT TGT GGA CAA AC 3'   (SEQ ID NO:7)
     3' TTC AGG AAT GAC AAC CAG GC 5'   (SEQ ID NO:8).

22. An isolated polypeptide forming a eag-like potassium
     channel having the characteristic of slowly activated
     outward rectification selected from the group consisting
     of *elk*1, *elk*2 and *eag*2.

23. The isolated polypeptide according to claim 21 forming a
     eag-like potassium channel for which the activation time
     constant for outward rectification is

24. The isolated polypeptide according to claim 21 wherein
     the potassium channel has an activation time constant for
     outward rectification of 676 ± 37 ms at 0 mV with a
     threshold activation at -40 mV at about pH 7.

25. The isolated polypeptide of claim 21, wherein the
     polypeptide is rat elk1.

26. The isolated polypeptide of claim 21, wherein the
     polypeptide is human elk1.

27.   The isolated polypeptide of claim 21, wherein the polypeptide is human elk2.

28.   The isolated polypeptide of claim 21, wherein the polypeptide is human eag2.

29.   The isolated polypeptide of claim 21 wherein the amino acid sequence of the polypeptide is SEQ ID NO:2.

30.   The isolated polypeptide of claim 21 wherein the partial amino acid sequence of the polypeptide is SEQ ID NO:3.

31.   The isolated polypeptide of claim 21 wherein the partial amino acid sequence of the polypeptide is SEQ ID NO:4.

32.   An expression vector comprising the nucleic acid of claim 1.

33.   An expression vector comprising the nucleic acid of claim 4.

34.   An expression vector comprising the nucleic acid of claim 5.

35.   An expression vector comprising the nucleic acid of claim 6.

36.   An expression vector comprising the nucleic acid of claim 7.

37.   An expression vector comprising the nucleic acid of claim 16.

38.   An expression vector comprising the nucleic acid of claim 19.

39.   A host cell transfected with the vector of claim 31.

40.   A host cell transfected with the vector of claim 32.

41.   A host cell transfected with the vector of claim 33.

42.   A host cell transfected with the vector of claim 34.

43.   A host cell transfected with the vector of claim 35.

44.   A host cell transfected with the vector of claim 36.

45.   A host cell transfected with the vector of claim 37.

46. A method for identifying a compound that modulates ion
     flux through a slowly activated outward rectifier
     potassium channel selected from the group consisting of
     elk1, elk2, eag2, the method comprising the steps of:

5   (i) contacting the compound with a eukaryotic host cell
     or cell membrane in which has been expressed a
     polypeptide forming a potassium channel having the
     characteristic of slowly activated outward rectification;
     and

10   (ii) determining the functional effect of the compound
     upon the cell or cell membrane expressing the potassium
     channel.

47. The method of claim 38, wherein the eukaryotic host cell
     is *Xenopus* oocyte.

15 48. The method of claim 38, wherein the functional effect is
     determined by measuring changes in current or voltage.

49. The method of claim 38, wherein the potassium channel
     polypeptide is recombinant.

50. The method of claim 38, wherein the potassium channel is

20   heteromeric.

51. The method of claim 38, wherein the potassium channel
     polypeptide is human elk1.

52. The method of claim 38, wherein the potassium channel
     polypeptide has an amino acid sequence of SEQ ID No:2.

25 53. A method of detecting the presence of elk1 in mamalian
     tissue selected from the group consisting of superior
     cervical ganglia, coeliac ganglia, superior mesenteric
     ganglia, foetal brain, adrenal and stellate ganglia, the
     method comprising the steps of

30   (i) isolating a biological sample from the mammalian
     tissue,

         (ii) contacting the biological sample with a elk1

specific reagent that selectively associates with elk1;
and

(iii) detecting the level of elk1 specific reagent that
selectively associates with the sample.

5   54. The method of claim 52, wherein the elk1-specific reagent
          is selected from the group consisting of: elk1 specific
          oligonucleotide primers, and elk1 nucleic acid probes.

     55. In a computer system, a method of screening for mutations
          of human *eag*-like genes selected from the group

10   consisting of *elk1*, *elk2* and *eag2*, the method comprising
          the steps of:

          (i)   entering into the computer system a first nucleic
          acid sequence encoding a slowly activated outward
          rectifier potassium channel polypeptide, said first

15   nucleic acid sequence having a nucleotide sequence
          selected from the group consisting of of SEQ ID No:1,
          comprising partial SEQ ID NO:13, and partial SEQ ID NO:
          14 and conservatively modified versions thereof;

          (ii)   comparing the first nucleic acid sequence with a

20   second nucleic acid sequence having substantial identity
          to the first nucleic acid sequence; and

          (iii) identifying nucleotide differences between the
          first and second nucleic acid sequences.

     56. The method of claim 54, wherein the second nucleic acid

25   sequence is associated with a disease state.

     57. A computer readable substrate comprising the first amino
          acid sequence of claim 24.

     58. A computer readable substrate comprising the first amino
          acid sequence of claim 25.

30   59. A computer readable substrate comprising the first amino
          acid sequence of claim 26.

60. A computer readable substrate comprising the first amino acid sequence of claim 27.

61. In a computer system, a method for identifying a three-dimensional structure of EAG-like polypeptides selected from the group consisting of elk1, elk2, eag2, the method comprising the steps of:

   (i) entering into the computer system an amino acid sequence of at least 10 amino acids of a potassium channel peptide or at least 30 nucleotides of a gene encoding the poypeptide, the polypeptide having a partial amino acid sequence selected from a group consisting of a part of SEQ ID No: 2, SEQ ID NO:3, SEQ ID NO:4 and conservatively modified versions thereof; and

   (ii) generating a three-dimensional structure of the polypeptide encoded by the amino-acid sequence.

62. The method of claim 60, wherein said amino acid sequence is a primary structure and wherein said generating step includes the steps of:

   (i) forming a secondary structure from said primary structure using energy terms determined by the primary structure; and

   (ii) forming a tertiary structure from said secondary structure using energy terms determined by said secondary structure.

63. The method of claim 61, wherein said generating step further includes the step of forming a quaternary structure from said tertiary structure using anisotrophic terms encoded by the tertiary structure.

64. The method of claim 60, further comprising the step of identifying; regions of the three-dimensional structure of the protein that bind to ligands and using the regions to identify ligands that bind to the polypeptide.

65. A computer readable substrate comprising the three dimensional structure of the polypeptide of claim 24.

66. A computer readable substrate comprising the three dimensional structure of the polypeptide of claim 25.

5 67. A computer readable substrate comprising the three dimensional structure of the polypeptide of claim 26.

68. A computer readable substrate comprising the three dimensional structure of the polypeptide of claim 27.
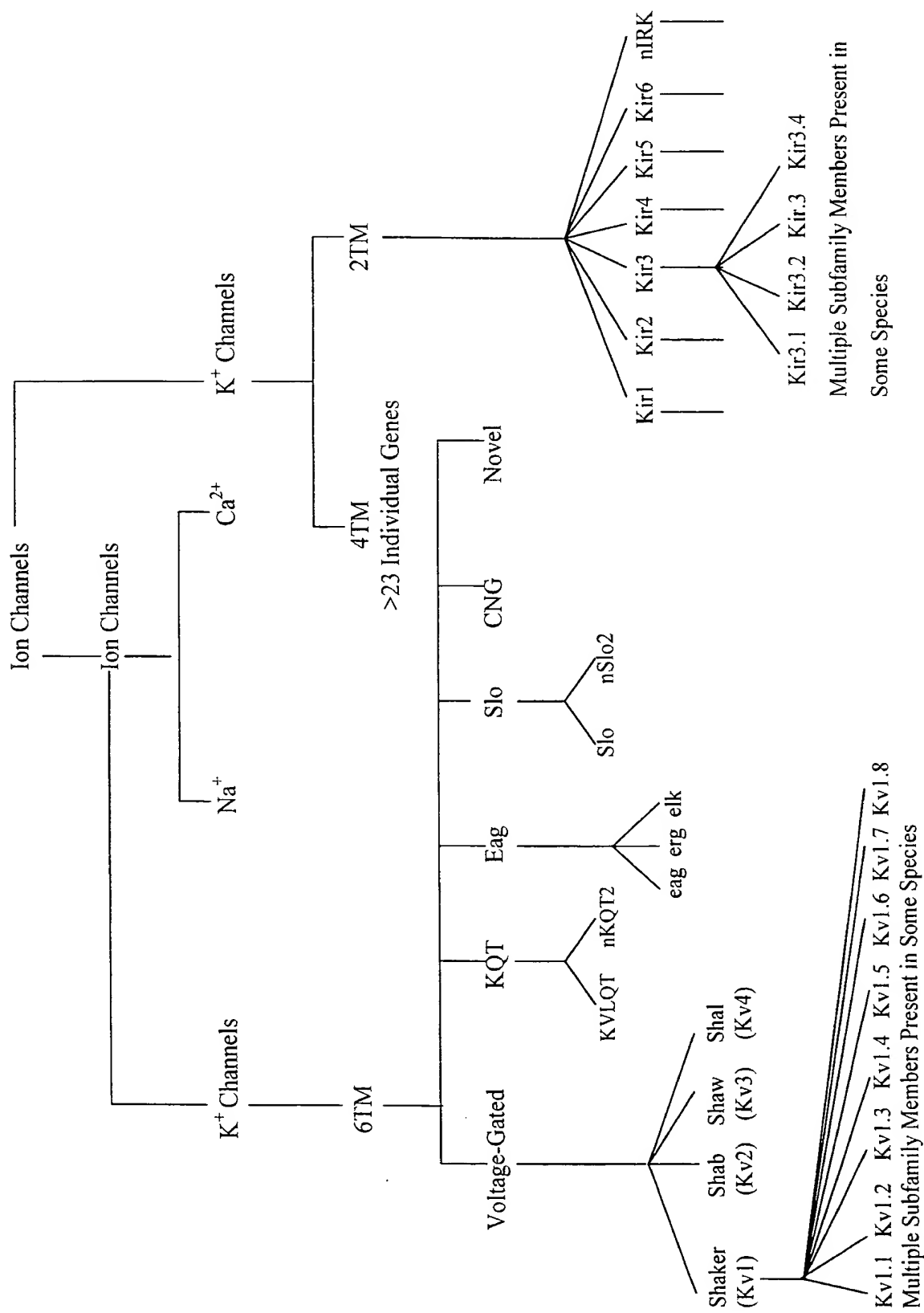
Fig. 1

Fig. 2

```
elk1    ---MPVMKGLLAPQNTFLDTIATRFDGTHSNFILANAQVAKGFPIVYCSD     47
erg1    ---MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVEN-CAVIYCND     46
eag1    MTMAGGRRGLVAPQNTFLENIVRRS--NDTNFVLGNAQIVD-WPIVYSND     47


elk1    GFCELAGFARTEVMQKSCSCKFLFGVETNEQLMLQIEKSLEEKVEFKGEI     97
erg1    GFCELCGYSRAEVMQRPCTCDFLHGPRTQRRAAAQIAQALLGAEERKVEI     96
eag1    GFCKLSGYHRAEVMQKSSACSFMYGELTDKDTVEKVRQTFENYEMNSFEI     97


elk1    MFYKKNGAPFWCLLDIVPIKNEKGDVVLFLASFKDITDTKVKITSEDKKE    147
erg1    AFYRKDGSCFLCLVDVVPVKNEDGAVIMFILNFEVVMEKDMVGSPAHDTN    146
eag1    LMYKKNRTPVWFFVKIAPIRNEQDKVVLFLCTFSDITA--FK-QPIKDDS    144


elk1    DRAK-----------------------GRSR------------------A    156
erg1    HRGPSTSWLASGRAKTFRLKLPALLALTARESPMRTGSTGSPGAPGAVVV    196
eag1    CKG-------------------WGKFAR----------------------    153


elk1    GSHFDSARRRS----------R----------------------------    168
erg1    DVDLTPAAPSSESLALDEVSAMDNHVAGLGPAEERRALVGPASASPVASI    246
eag1    ---LTRALTSS----------R-------G--------------------    163


elk1    ------AVLYHISGHLQ---------------------------------    179
erg1    PGPHPSPRAQSLNPDASGSSCSLARTRSRESCASVRRASSADDIEAMRAG    296
eag1    -------VLQQLAPSVQ---------------------------------    173


elk1    --------------------------------------------------    179
erg1    ALPLPPRHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLK    346
eag1    -------------------------------------------------K    174


elk1    ------------------RREKNK---LKINNNVFVDKPAFPEYKVSDAK    208
erg1    GDPFLASPTSDREIIAPKIKERTHNVTEKVTQVLSLGADVLPEYKLQAPR    396
eag1    G-----------------ENVHKH---SRLAEVLQLGSDILPQYKQEAPK    204


               ----------S1----------
elk1    KSKFILLHFSTFKAGWDWLILLATFYVAVTVPYNVCFIGNED--LS----    252
erg1    IHRWTILHYSPFKAVWDWLILLLVIYTAVFTPYSAAFLLKETEDGSQAPD    446
eag1    PPHIILHYCVFKTTTWDWIILILTFYTAILVPYNVSFKTRQN-N------    247


               ----------S2----------              --
elk1    ---TTRSTTVSDIAVEILFIIDIILNFRTTYVSKSGQVIFEARSICIHYV    299
erg1    CGYACQPLAVVDLLVDIMFIVDILINFRTTYVNANEEVVSHPGRIAVHYF    496
eag1    -----VAWLVVDSIVDVIFLVDIVLNFHTTFVGPAGEVISDPKLIRMNYL    292
```

Fig. 2(Cont'd)

```
     ------S3---------              ------------S4-------
elk1 TTWFIIDLIAALPFDLLYAFNVTVVS---LVHLLKTVRLLRLLRLLQKLD     346
erg1 KGWFLIDMVAAIPFDLLIFGSGSEE----LIGLLKTARLLRLVRVARKLD     542
eag1 KTWFVIDLLSCLPYDVINAFENVDEGISSLFSSLKVVRLLRLGRVARKLD     342


     -       ---------S5----------
elk1 RYSQHSTIVLTLLMSMFALLAHWMACIWYVIGKMEREDNSLLKWEVG-WL     395
erg1 RYSEYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSHIG----WL     588
eag1 HYIEYGAAVLVLLVCVFGLAAHWMACIWYSIGDYEIFDEDTKTIRNNSWL     392


     ---------------------P------------------------
elk1 HELGKRLESPYYGNN-----TLGGPSIRSAYIAALYFTLSSLTSVGFGNV     440
erg1 HNLGDQIGKPYNSSG------LGGPSIKDKYVTALYFTFSSLTSVGFGNV     632
eag1 YQLALDIGTPYQFNGSGSGKWEGGPSKNSVYISSLYFTMTSLTSVGFGNI     442


     ----------S6-------------
elk1 SANTDAEKIFSICTMLIGALMHALVFGNVTAIIQRMYSRWSLYHTRTKDL     490
erg1 SPNTNSEKIFSICVMLIGSLMYASIFGNVSAIIQRLYSGTARYHTQMLRV     682
eag1 APSTDIEKIFAVAIMMIGSLLYATIFGNVTTIFQQMYANTNRYHEMLNSV     492


elk1 KDFIRVHHLPQQLKQRMLEYFQTTWSVNNGIDSNELLKDFPDELRSDITM     540
erg1 REFIRFHQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGFPECLQADICL     732
eag1 RDFLKLYQVPKGLSERVMDYIVSTWSMSRGIDTKKVLQICPKDNRADICV     542


                                ------------------
elk1 HLNKEILQLS-LFECASRGCLRSLSLHIKTSFCAPGEYLLRQGDALQAIY     589
erg1 HLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALY     782
eag1 HLNRKVFKEHPAFRLASDGCLRALAMEFQTVHCAPGDLIYHAGEDVDSLC     592


     ----------cNBD----------------------------------
elk1 FVCSGSMEVLKDSMVLAILGKGDLIGANLSIKDQVIKTNADVKALTYCDL     639
erg1 FISRGSIEILRGDVVVAILGKNDIFGEPLNLYARPGKSNGDVRALTYCDL     832
eag1 FVVSGSLEVIQDDEVVAILGKGDVFGDVFWKEATLAQSCANVRALTYCDL     642


elk1 QCIILKGLFEVLGLYPEYAHKFVEDIQHDLTYNLREGHESDVISRLSNKS     689
erg1 HKIHRDDLLEVLDMYPEFSDHFWS--SLEITFNLRDTNMIPG--------     872
eag1 HVIKRDALQKVLEFYTAFSHSFSR--NLILTYNLRKRIVFR---------     681


elk1 TVPQAEPKGNGSIKKRLPSIVEDEEEEEVEEEETTSLSPIYTRGSSVSHS     739
erg1 ----------------SPSSAELESGFNRQ------------RKRKLSFR     894
eag1 ---------------KISDVKREEEERMK------------RKNEAPLI     703
```

Fig. 2 (Cont'd)

```
elk1    KKTGSSKSYLGLSLKQLTSGTVPFHSPIRVSSANSPKTKQEADPPNHGTR      789
erg1    RRTDKDTEQPGEVSALG-------QGPARVGPGPSCRG-QPGGPWGESP-      935
eag1    LPPDHPVRRLFQRFRQ-------QKEARLAAE---RGGRDLDDLDVEK-      741


elk1    KEKNLKVQLCSLGTAGTPELSPRIVDGIEDGNSSEETQTFDFGSEQIRPE      839
erg1    ------------SSGPSSPES----------SEDEGPGRSSSPLRLVPF      962
eag1    ------------GNALTDHTS----------ANHSLVKASVVTVRESPA      768


elk1    PRISPSLGESEIGAAFLFIKAEETKQQINKLNSEVTTLTQEVSQLGKDMR      889
erg1    S--SPRPPGDSPGGEPLTEDGEKSSDTCNPLSGAFSGVSN-IFSFWGDSR     1009
eag1    TPVSFQAASTSTVSDHAKLHAP-GSECLGPKAGGGDPAKRKGWARFKDAC      817


elk1    SI-MQLLENILSPQQPSQFCSLHPTSICPSRESFQTRVSWSAHQPCLHLQ      938
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eag1    GK--GEDWNKVSK-------------AESMETLPERTKASGEATLKKTD      851


elk1    ANGAHLYHGNVTSDIWSVDPSLVGSNPQRTEAHEQSPVDSELHHSPNLAY      988
erg1    GD---------VESRLDALQRQDNRLETRLSAD-MATVLQLLQRQMTLVP     1079
eag1    SC---------DSGITKSDLRLDNVGEAPSPQD-RSPILAEVKHSFYPIP      891


elk1    SPSHCQVIQEGHLQFLRCISPHSDTTLTPLQSISATLSSSVCSSSETSLH     1038
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eag1    EQTLQATVLE----------VKHELKEDIKALNAKMTSIEKQLSEILRI      930


elk1    LVLPSRSEEGSITHGPVSSFSLENLPGSWDREGMMSASTEPLENFPVEVV     1088
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eag1    LMSR-----GSSQSPQD--ICEVSRP--Q---------S-PESDRDIFGA      961


elk1    TSTADVKDSKAINV      1102
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Fig 3

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Fig. 3 (Cont'd)

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Fig. 3 (Cont'd)

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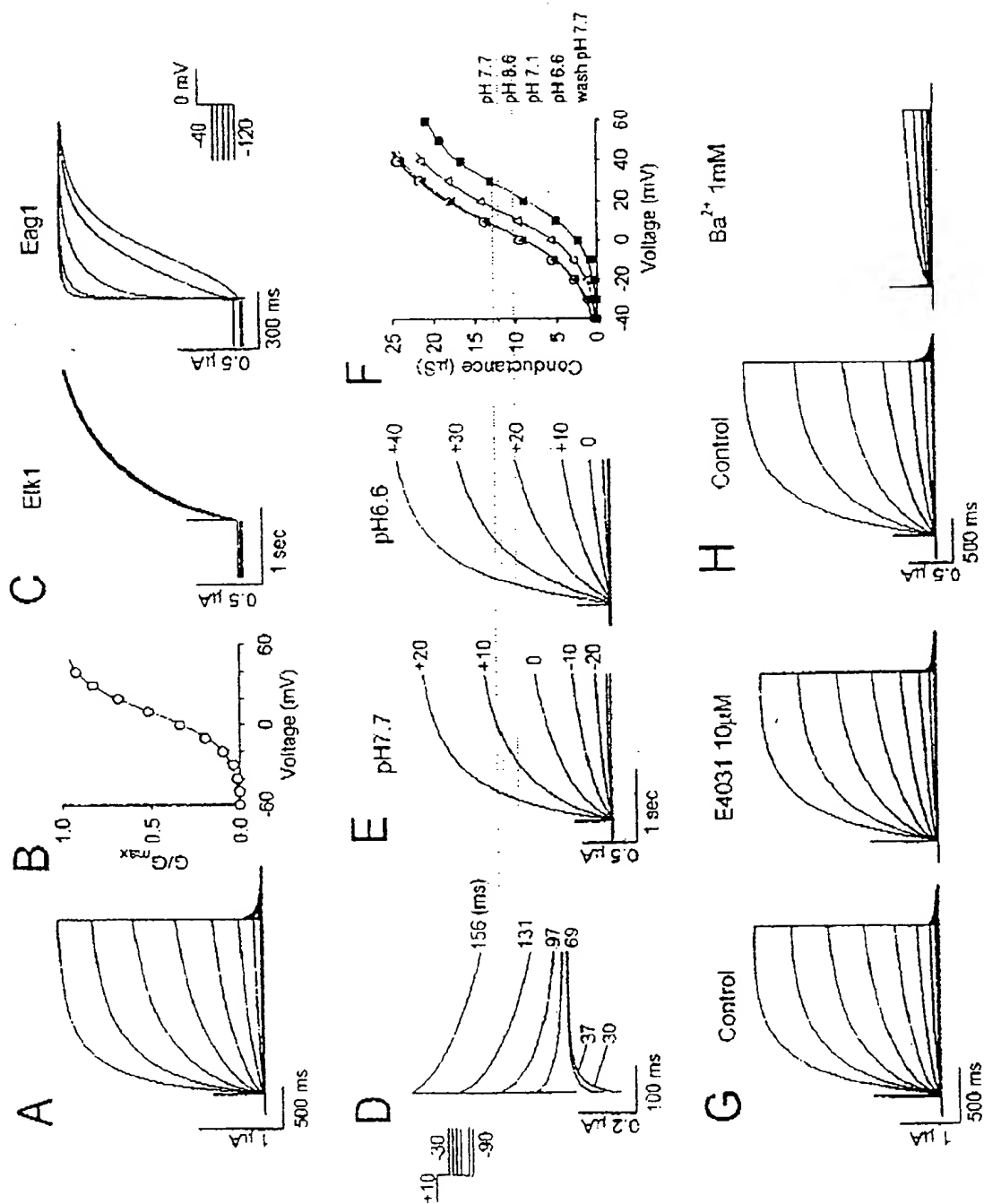
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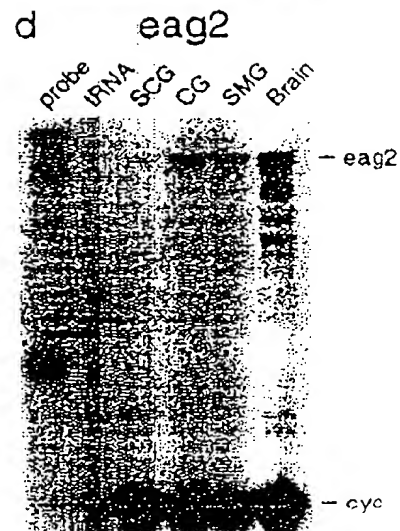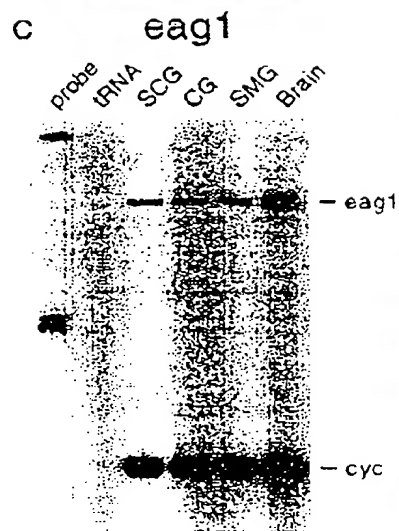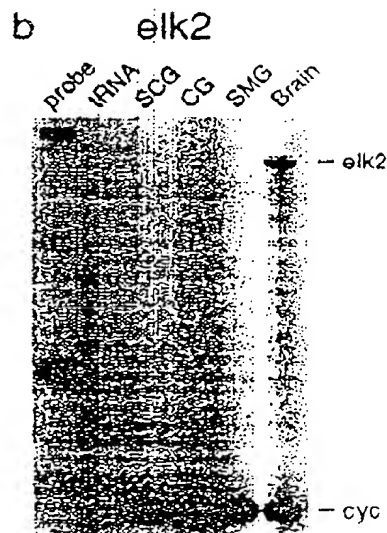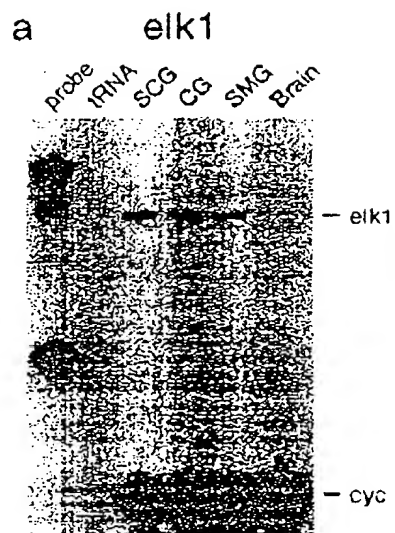
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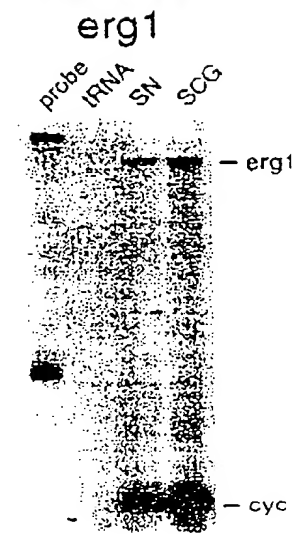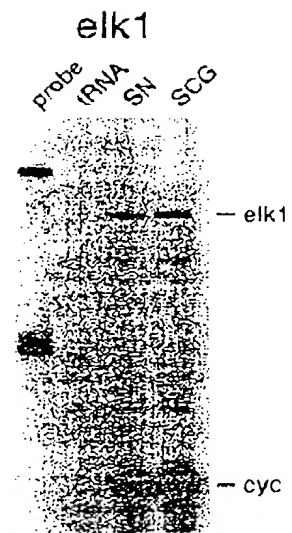
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  65   70   75   80

Pro Asn Leu Pro Glu Tyr Lys Val Ala Ala Ile Arg Lys Ser Pro Phe
                85   90   95

Ile Leu Leu His Cys Gly Ala Leu Arg Ala Thr Trp Asp Gly Phe Ile
            100   105   110

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Leu Leu Ala Thr Leu Tyr Val Ala Val Thr Val Pro Tyr Ser Val Cys
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Ser Val Val Asp Val Ile Phe Leu Val Asp Ile Val Leu Asn Phe His
        35                  40                  45

Thr Thr Phe Val Gly Pro Gly Gly Glu Val Ile Ser Asp Pro Lys Leu
        50                  55                  60

Ile Arg Met Asn Tyr Leu Lys Thr Trp Phe Val Ile Asp Leu Leu Ser
 65                 70                  75                  80

Cys Leu Pro Tyr Asp Ile Ile Asn Ala Phe Glu Asn Val Asp Glu Gly
                85                  90                  95

Ile Ser Ser Leu Phe Ser Ser Leu Lys Val Val Arg Leu Leu Arg Leu
            100                 105                 110

Gly Arg Val Ala Arg Lys Leu Asp His Tyr Leu Glu Tyr Gly Ala Ala
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tccaaaggct ttaatgccaa tcggaggcgc agccgggcgg ttctctacca cctctctggt 180
cacctgcaga aacaacccaa gggcaagcac aaactcaata agggtgtgtt tggagagaag 240
ccaaatttgc ccgaatataa agtcgctgct atccggaagt caccctttat cctgctgcac 300
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gtggacatcg ttttaaactt tcacacgact tttgtggggc cgggtggaga ggtcatttct 180
gacccaaaac tcatacggat gaactatctg aaaacttggt ttgtgattga tctgctgtct 240
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11

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                          20                    25                    30

           Asn Ala Arg Val Glu Asn Cys Ala Val Ile Tyr Cys Asn Asp Gly Phe
                     35                    40                    45

           Cys Glu Leu Cys Gly Tyr Ser Arg Ala Glu Val Met Gln Arg Pro Cys
                     50                    55                    60

           Thr Cys Asp Phe Leu His Gly Pro Arg Thr Gln Arg Arg Ala Ala Ala
           65                    70                    75                    80

           Gln Ile Ala Gln Ala Leu Leu Gly Ala Glu Glu Arg Lys Val Glu Ile
                          85                    90                    95

           Ala Phe Tyr Arg Lys Asp Gly Ser Cys Phe Leu Cys Leu Val Asp Val
                          100                   105                   110

           Val Pro Val Lys Asn Glu Asp Gly Ala Val Ile Met Phe Ile Leu Asn
                     115                   120                   125

           Phe Glu Val Val Met Glu Lys Asp Met Val Gly Ser Pro Ala His Asp
                     130                   135                   140

           Thr Asn His Arg Gly Pro Ser Thr Ser Trp Leu Ala Ser Gly Arg Ala
           145                   150                   155                   160

           Lys Thr Phe Arg Leu Lys Leu Pro Ala Leu Leu Ala Leu Thr Ala Arg
                          165                   170                   175

           Glu Ser Pro Met Arg Thr Gly Ser Thr Gly Ser Pro Gly Ala Pro Gly
                          180                   185                   190

           Ala Val Val Val Asp Val Asp Leu Thr Pro Ala Ala Pro Ser Ser Glu
                     195                   200                   205

           Ser Leu Ala Leu Asp Glu Val Ser Ala Met Asp Asn His Val Ala Gly
                210                   215                   220

           Leu Gly Pro Ala Glu Glu Arg Arg Ala Leu Val Gly Pro Ala Ser Ala
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                          245                   250                   255

           Ser Leu Asn Pro Asp Ala Ser Gly Ser Ser Cys Ser Leu Ala Arg Thr
```

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Asp Ile Glu Ala Met Arg Ala Gly Ala Leu Pro Leu Pro Pro Arg His
    290                     295                     300

Ala Ser Thr Gly Ala Met His Pro Leu Arg Ser Gly Leu Leu Asn Ser
305                     310                     315                     320

Thr Ser Asp Ser Asp Leu Val Arg Tyr Arg Thr Ile Ser Lys Ile Pro
                325                     330                     335

Gln Ile Thr Leu Asn Phe Val Asp Leu Lys Gly Asp Pro Phe Leu Ala
            340                     345                     350

Ser Pro Thr Ser Asp Arg Glu Ile Ile Ala Pro Lys Ile Lys Glu Arg
        355                     360                     365

Thr His Asn Val Thr Glu Lys Val Thr Gln Val Leu Ser Leu Gly Ala
    370                     375                     380

Asp Val Leu Pro Glu Tyr Lys Leu Gln Ala Pro Arg Ile His Arg Trp
385                     390                     395                     400

Thr Ile Leu His Tyr Ser Pro Phe Lys Ala Val Trp Asp Trp Leu Ile
                405                     410                     415

Leu Leu Leu Val Ile Tyr Thr Ala Val Phe Thr Pro Tyr Ser Ala Ala
            420                     425                     430

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        435                     440                     445

Tyr Ala Cys Gln Pro Leu Ala Val Val Asp Leu Leu Val Asp Ile Met
    450                     455                     460

Phe Ile Val Asp Ile Leu Ile Asn Phe Arg Thr Thr Tyr Val Asn Ala
465                     470                     475                     480

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                485                     490                     495

Lys Gly Trp Phe Leu Ile Asp Met Val Ala Ala Ile Pro Phe Asp Leu
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Leu Ile Phe Gly Ser Gly Ser Glu Glu Leu Ile Gly Leu Leu Lys Thr
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                    515   520   525

Ala Arg Leu Leu Arg Leu Val Arg Val Ala Arg Lys Leu Asp Arg Tyr
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Leu Ile Ala His Trp Leu Ala Cys Ile Trp Tyr Ala Ile Gly Asn Met
                565   570   575

Glu Gln Pro His Met Asp Ser His Ile Gly Trp Leu His Asn Leu Gly
                580   585   590

Asp Gln Ile Gly Lys Pro Tyr Asn Ser Ser Gly Leu Gly Gly Pro Ser
                595   600   605

Ile Lys Asp Lys Tyr Val Thr Ala Leu Tyr Phe Thr Phe Ser Ser Leu
    610   615   620

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625   630   635   640

Ile Phe Ser Ile Cys Val Met Leu Ile Gly Ser Leu Met Tyr Ala Ser
                645   650   655

Ile Phe Gly Asn Val Ser Ala Ile Ile Gln Arg Leu Tyr Ser Gly Thr
                660   665   670

Ala Arg Tyr His Thr Gln Met Leu Arg Val Arg Glu Phe Ile Arg Phe
    675   680   685

His Gln Ile Pro Asn Pro Leu Arg Gln Arg Leu Glu Glu Tyr Phe Gln
    690   695   700

His Ala Trp Ser Tyr Thr Asn Gly Ile Asp Met Asn Ala Val Leu Lys
705   710   715   720

Gly Phe Pro Glu Cys Leu Gln Ala Asp Ile Cys Leu His Leu Asn Arg
                725   730   735

Ser Leu Leu Gln His Cys Lys Pro Phe Arg Gly Ala Thr Lys Gly Cys
                740   745   750

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Asp Thr Leu Val His Ala Gly Asp Leu Leu Thr Ala Leu Tyr Phe Ile

14

                    770   775   780

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Leu Gly Lys Asn Asp Ile Phe Gly Glu Pro Leu Asn Leu Tyr Ala Arg
                805   810   815

Pro Gly Lys Ser Asn Gly Asp Val Arg Ala Leu Thr Tyr Cys Asp Leu
                820   825   830

His Lys Ile His Arg Asp Asp Leu Leu Glu Val Leu Asp Met Tyr Pro
            835   840   845

Glu Phe Ser Asp His Phe Trp Ser Ser Leu Glu Ile Thr Phe Asn Leu
            850   855   860

Arg Asp Thr Asn Met Ile Pro Gly Ser Pro Ser Ser Ala Glu Leu Glu
865   870   875   880

Ser Gly Phe Asn Arg Gln Arg Lys Arg Lys Leu Ser Phe Arg Arg Arg
                885   890   895

Thr Asp Lys Asp Thr Glu Gln Pro Gly Glu Val Ser Ala Leu Gly Gln
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Gly Pro Ala Arg Val Gly Pro Gly Pro Ser Cys Arg Gly Gln Pro Gly
                915   920   925

Gly Pro Trp Gly Glu Ser Pro Ser Ser Gly Pro Ser Ser Pro Glu Ser
            930   935   940

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                980   985   990

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                995   1000   1005

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                1045   1050   1055

Glu Thr Arg Leu Ser Ala Asp Met Ala Thr Val Leu Gln Leu Leu Gln
                1060   1065   1070

Arg Gln Met Thr Leu Val Pro Pro Ala Tyr Ser Ala Val Thr Thr Pro
            1075   1080   1085

Gly Pro Gly Pro Thr Ser Thr Ser Pro Leu Leu Pro Val Gly Pro Val
            1090   1095   1100

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1105   1110   1115   1120

Phe Glu Glu Leu Pro Ala Gly Ala Pro Glu Leu Pro Gln Asp Gly Pro
                1125   1130   1135

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    50   55   60

Ser Ala Cys Ser Phe Met Tyr Gly Glu Leu Thr Asp Lys Asp Thr Val
65   70   75   80

Glu Lys Val Arg Gln Thr Phe Glu Asn Tyr Glu Met Asn Ser Phe Glu
                85   90   95

Ile Leu Met Tyr Lys Lys Asn Arg Thr Pro Val Trp Phe Phe Val Lys
            100   105   110

Ile Ala Pro Ile Arg Asn Glu Gln Asp Lys Val Val Leu Phe Leu Cys
        115   120   125

Thr Phe Ser Asp Ile Thr Ala Phe Lys Gln Pro Ile Lys Asp Asp Ser
    130   135   140

Cys Lys Gly Trp Gly Lys Phe Ala Arg Leu Thr Arg Ala Leu Thr Ser
145   150   155   160

Ser Arg Gly Val Leu Gln Gln Leu Ala Pro Ser Val Gln Lys Gly Glu
                165   170   175

Asn Val His Lys His Ser Arg Leu Ala Glu Val Leu Gln Leu Gly Ser
            180   185   190

Asp Ile Leu Pro Gln Tyr Lys Gln Glu Ala Pro Lys Pro Pro His Ile
        195   200   205

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Leu Ile Leu Thr Phe Tyr Thr Ala Ile Leu Val Pro Tyr Asn Val Ser
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Phe Lys Thr Arg Gln Asn Asn Val Ala Trp Leu Val Val Asp Ser Ile
                245   250   255

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            260   265   270

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        275   280   285

Met Asn Tyr Leu Lys Thr Trp Phe Val Ile Asp Leu Leu Ser Cys Leu
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Ser Leu Phe Ser Ser Leu Lys Val Val Arg Leu Leu Arg Leu Gly Arg
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            420   425   430

Ser Leu Thr Ser Val Gly Phe Gly Asn Ile Ala Pro Ser Thr Asp Ile
            435   440   445

Glu Lys Ile Phe Ala Val Ala Ile Met Met Ile Gly Ser Leu Leu Tyr
    450   455   460

Ala Thr Ile Phe Gly Asn Val Thr Thr Ile Phe Gln Gln Met Tyr Ala
465   470   475   480

Asn Thr Asn Arg Tyr His Glu Met Leu Asn Ser Val Arg Asp Phe Leu
            485   490   495

Lys Leu Tyr Gln Val Pro Lys Gly Leu Ser Glu Arg Val Met Asp Tyr
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Ile Val Ser Thr Trp Ser Met Ser Arg Gly Ile Asp Thr Lys Lys Val
            515   520   525

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            660                 665                 670

Asn Leu Arg Lys Arg Ile Val Phe Arg Lys Ile Ser Asp Val Lys Arg
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Leu Asp Val Glu Lys Gly Asn Ala Leu Thr Asp His Thr Ser Ala Asn
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Thr Pro Val Ser Phe Gln Ala Ala Ser Thr Ser Thr Val Ser Asp His
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Ala Lys Leu His Ala Pro Gly Ser Glu Cys Leu Gly Pro Lys Ala Gly
785                 790                 795                 800

Gly Gly Asp Pro Ala Lys Arg Lys Gly Trp Ala Arg Phe Lys Asp Ala
            805                 810                 815

Cys Gly Lys Gly Glu Asp Trp Asn Lys Val Ser Lys Ala Glu Ser Met
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Lys Thr Asp Ser Cys Asp Ser Gly Ile Thr Lys Ser Asp Leu Arg Leu
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Asp Asn Val Gly Glu Ala Pro Ser Pro Gln Asp Arg Ser Pro Ile Leu
865   870   875   880

Ala Glu Val Lys His Ser Phe Tyr Pro Ile Pro Glu Gln Thr Leu Gln
            885   890   895

Ala Thr Val Leu Glu Val Lys His Glu Leu Lys Glu Asp Ile Lys Ala
        900   905   910

Leu Asn Ala Lys Met Thr Ser Ile Glu Lys Gln Leu Ser Glu Ile Leu
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Arg Ile Leu Met Ser Arg Gly Ser Ser Gln Ser Pro Gln Asp Ile Cys
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Glu Val Ser Arg Pro Gln Ser Pro Glu Ser Asp Arg Asp Ile Phe Gly
945   950   955   960

Ala Ser

INTERNATIONAL SEARCH REPORT

International application No.
US99/19902

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07K 14/00, 14/435; C12N 5/16, 15/12, 15/63; G01N 33/53, 33/566
US CL :435/6, 7.2, 320.1, 325; 530/350; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.2, 320.1, 325; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, BIOSCIENCE, MEDLINE, BIOSIS, CAPLUS, GENESEQ, GENBANK, PIR

search terms: potassium channel, ether-a-go-go, eag, erg, elk

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	SHI et al. Identification of two nervous system-specific members of the *erg* potassium channel gene family. The Journal of Neuroscience. 15 December 1997, Vol. 17, No. 24, pages 9423-9432, especially pages 9423-9426 and figures 1-8.	1-10, 12-15, 17-22, 32-36, 38, 40-44, 46, 53 --------- 11, 16, 37, 45

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

<table>
<tr><td>*</td><td>Special categories of cited documents:</td><td>"T"</td><td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr>
<tr><td>"A"</td><td>document defining the general state of the art which is not considered to be of particular relevance</td><td></td><td></td></tr>
<tr><td>"E"</td><td>earlier document published on or after the international filing date</td><td>"X"</td><td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr>
<tr><td>"L"</td><td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>"Y"</td><td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr>
<tr><td>"O"</td><td>document referring to an oral disclosure, use, exhibition or other means</td><td></td><td></td></tr>
<tr><td>"P"</td><td>document published prior to the international filing date but later than the priority date claimed</td><td>"&"</td><td>document member of the same patent family</td></tr>
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Date of the actual completion of the international search 12 NOVEMBER 1999	Date of mailing of the international search report **25 JAN 2000**
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL PAK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/19902

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 23-31, 39, 47-52, 54-68
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

   Please See Extra Sheet.

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)★

INTERNATIONAL SEARCH REPORT

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

claims 23-31 are dependent on claim 21 which is not directed to the isolated polypeptide but to an isolated polynucleotide. Claim 39 is dependent on claim 31 which is not directed to the vector. Claims 47-52, 54, and 56 are directed to method of claim 38 but claim 38 is a vector. Claims 57-60 are directed amino acid sequences of claims 24-27, but claims 24-27 are dependent on claim 21 which are not directed to isolated polypeptide. Claims 55 and 61 does not clearly define whether the invention is directed to a computer system or a method and claim 63 is directed to claim 61 which is unsearchable. Claims 62 and 64 are dependent on a method of claim 60 but claim 60 is directed to a computer readable substrate and not a method. Claims 65-68 are dependent on claims 24-27 which are unsearchable because the claims 24-27 are dependent on claim 21 which are directed to isolated nucleic acid but not polypeptides.